considered on 3/21/03 MCS

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=> fil capl; d que 13; d que 15; s l3 or l5
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FILE COVERS 1907 - 27 Dec 2002  VOL 138 ISS 1
FILE LAST UPDATED: 26 Dec 2002  (20021226/ED)

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L1            17 SEA FILE=CAPLUS ABB=ON  DELAGRAVE S?/AU
L2            67 SEA FILE=CAPLUS ABB=ON  MARRS B?/AU
L3             4 SEA FILE=CAPLUS ABB=ON  L1 AND L2


L1            17 SEA FILE=CAPLUS ABB=ON  DELAGRAVE S?/AU
L2            67 SEA FILE=CAPLUS ABB=ON  MARRS B?/AU
L4         17399 SEA FILE=CAPLUS ABB=ON  LIBRAR?/OBI
L5             6 SEA FILE=CAPLUS ABB=ON  (L1 OR L2) AND L4


L162           8 L3 OR L5

=> fil wpids; d que l42; fil biosis; d que l67

FILE 'WPIDS' ENTERED AT 16:27:16 ON 27 DEC 2002
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FILE LAST UPDATED:          20 DEC 2002     <20021220/UP>
MOST RECENT DERWENT UPDATE:     200282       <200282/DW>
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L40 2 SEA FILE=WPIDS ABB=ON DELAGRAVE S?/AU
L41 3 SEA FILE=WPIDS ABB=ON MARRS B?/AU
L42 4 SEA FILE=WPIDS ABB=ON L40 OR L41

FILE 'BIOSIS' ENTERED AT 16:27:17 ON 27 DEC 2002
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FILE COVERS 1969 TO DATE.
CAS REGISTRY NUMBERS AND CHEMICAL NAMES (CNs) PRESENT
FROM JANUARY 1969 TO DATE.

RECORDS LAST ADDED: 25 December 2002 (20021225/ED)

L65 7 SEA FILE=BIOSIS ABB=ON DELAGRAVE S?/AU
L66 86 SEA FILE=BIOSIS ABB=ON MARRS B?/AU
L67 1 SEA FILE=BIOSIS ABB=ON L65 AND L66

=> fil biotechno; d que l100; fil biotechds; d que l138

FILE 'BIOTECHNO' ENTERED AT 16:27:18 ON 27 DEC 2002
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FILE LAST UPDATED: 17 DEC 2002 <20021217/UP>
FILE COVERS 1980 TO DATE.

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L98 9 SEA FILE=BIOTECHNO ABB=ON DELAGRAVE S?/AU
L99 21 SEA FILE=BIOTECHNO ABB=ON MARRS B?/AU
L100 1 SEA FILE=BIOTECHNO ABB=ON L98 AND L99

FILE 'BIOTECHDS' ENTERED AT 16:27:18 ON 27 DEC 2002
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L136 6 SEA FILE=BIOTECHDS ABB=ON DELAGRAVE S?/AU
L137 5 SEA FILE=BIOTECHDS ABB=ON MARRS B?/AU
L138 1 SEA FILE=BIOTECHDS ABB=ON L136 AND L137

=> dup rem l162,l67,l100,l138,l42
FILE 'CAPLUS' ENTERED AT 16:27:51 ON 27 DEC 2002
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FILE 'BIOTECHNO' ENTERED AT 16:27:51 ON 27 DEC 2002

FILE 'BIOTECHDS' ENTERED AT 16:27:51 ON 27 DEC 2002

FILE 'WPIDS' ENTERED AT 16:27:51 ON 27 DEC 2002

PROCESSING COMPLETED FOR L162
PROCESSING COMPLETED FOR L67
PROCESSING COMPLETED FOR L100
PROCESSING COMPLETED FOR L138
PROCESSING COMPLETED FOR L42
L163 11 DUP REM L162 L67 L100 L138 L42 (4 DUPLICATES REMOVED)
ANSWERS '1-8' FROM FILE CAPLUS
ANSWER '9' FROM FILE BIOTECHDS
ANSWERS '10-11' FROM FILE WPIDS

=> d ibib ab 1-11

L163 ANSWER 1 OF 11 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 1
ACCESSION NUMBER: 2001:851412 CAPLUS
DOCUMENT NUMBER: 136:1559
TITLE: Methods for the enzymatic assembly of polynucleotides and identification of polynucleotides having desired characteristics
INVENTOR(S): **Delagrave, Simon; Marrs, Barry**
PATENT ASSIGNEE(S): Hercules Incorporated, USA
SOURCE: PCT Int. Appl., 60 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001088173	A2	20011122	WO 2001-US14966	20010510
WO 2001088173	A3	20020606		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 6479262	B1	20021112	US 2000-571774	20000516

PRIORITY APPLN. INFO.: US 2000-571774 A 20000516

AB The present invention provides methods of prepg. large polynucleotides of arbitrary sequence and in a manner that will readily lend itself to automation. The present invention provides methods of prepg. a polynucleotide having at least 200 nucleotides in either a 5' to 3' or 3' to 5' direction by ligating a plurality of oligonucleotides, the assembly of which, represents the nucleotide sequence of the desired polynucleotide. The present invention also provides libraries of polynucleotides and screening of libraries for polynucleotide members

having desired properties.

L163 ANSWER 2 OF 11 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 2
ACCESSION NUMBER: 2001:542804 CAPLUS
DOCUMENT NUMBER: 135:177049
TITLE: Application of a very high-throughput digital imaging screen to evolve the enzyme galactose oxidase
AUTHOR(S): **Delagrave, Simon**; Murphy, Dennis J.; Pruss, Jennifer L. Rittenhouse; Maffia, Anthony M., III; **Marrs, Barry L.**; Bylina, Edward J.; Coleman, William J.; Grek, Christina L.; Dilworth, Michael R.; Yang, Mary M.; Youvan, Douglas C.
CORPORATE SOURCE: Corporate Research, Hercules Incorporated, Wilmington, DE, 19808, USA
SOURCE: Protein Engineering (2001), 14(4), 261-267
CODEN: PRENE9; ISSN: 0269-2139
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Directed evolution has become an important enabling technol. for the development of new enzymes in the chem. and pharmaceutical industries. Some of the most interesting substrates for these enzymes, such as polymers, have poor soly. or form highly viscous solns. and are therefore refractory to traditional high-throughput screens used in directed evolution. We combined digital imaging spectroscopy and a new solid-phase screening method to screen enzyme variants on problematic substrates highly efficiently and show here that the specific activity of the enzyme galactose oxidase can be improved using this technol. One of the variants we isolated, contg. the mutation C383S, showed a 16-fold increase in activity, due in part to a 3-fold improvement in Km. The present methodol. should be applicable to the evolution of numerous other enzymes, including polysaccharide-modifying enzymes that could be used for the large-scale synthesis of modified polymers with novel chem. properties.
REFERENCE COUNT: 25 THERE ARE 25 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L163 ANSWER 3 OF 11 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 3
ACCESSION NUMBER: 1999:424502 CAPLUS
DOCUMENT NUMBER: 131:239489
TITLE: Novel approaches for discovering industrial enzymes
AUTHOR(S): **Marrs, Barry; Delagrave, Simon**; Murphy, Dennis
CORPORATE SOURCE: Hercules Research Center, Hercules Incorporated, Wilmington, DE, 19808, USA
SOURCE: Current Opinion in Microbiology (1999), 2(3), 241-245
CODEN: COMIF7; ISSN: 1369-5274
PUBLISHER: Current Biology Publications
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review with 40 refs. New technologies for enzyme discovery are changing the rules of the game for industrial biocatalysis. More kinds of enzymes are available, their hardiness is increasing, and their costs are coming down. These changes are the key drivers for a rebirth of interest in industrial applications of enzymes. The major enabling discovery approaches include screening of biodiversity, genomic sequencing, directed evolution and phage display.
REFERENCE COUNT: 40 THERE ARE 40 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L163 ANSWER 4 OF 11 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 4
ACCESSION NUMBER: 1997:195785 CAPLUS
DOCUMENT NUMBER: 126:183161
TITLE: Recombinant enzyme **libraries**, process for

screening the **libraries**, and enzyme kits
INVENTOR(S): Short, Jay M.; **Marrs, Barry**; Stein, Jeffrey L.
PATENT ASSIGNEE(S): Recombinant Biocatalysis, Inc., USA; Short, Jay M.; Marrs, Barry; Stein, Jeffrey L.
SOURCE: PCT Int. Appl., 68 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 31
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9704077	A1	19970206	WO 1996-US11854	19960717
W: AU, CA, IL, JP, US, US, US				
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
US 6004788	A	19991221	US 1995-503606	19950718
US 5958672	A	19990928	US 1996-657409	19960603
EP 839185	A1	19980506	EP 1996-925351	19960717
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2002514894	T2	20020521	JP 1997-506829	19960717
US 6168919	B1	20010102	US 1998-983367	19980930
US 2002058254	A1	20020516	US 2001-753752	20010102
US 2002051987	A1	20020502	US 2001-861267	20010518
US 2002086279	A1	20020704	US 2001-875412	20010606

PRIORITY APPLN. INFO.:

US 1995-503606	A	19950718
US 1995-568994	A	19951207
US 1996-657409	A	19960603
WO 1996-US11854	W	19960717
US 1997-988224	A1	19971210
US 1998-983367	A3	19980930
US 1999-467740	A3	19991220

AB Recombinant enzyme libraries and kits where a plurality of enzymes are each characterized by different phys. and/or chem. characteristics and classified by common characteristics are disclosed. The characteristics are detd. by screening of recombinant enzymes expressed by a DNA library produced from various microorganisms. Also disclosed is a process for identifying clones of a recombinant library which expresses a protein with a desired activity by screening a library of expression clones randomly produced form DNA of at least one microorganism, said screening being effected on expression products of said clones to thereby identify clones which express a protein with a desired activity. Also disclosed is a process of screening clones having DNA from an uncultivated microorganism for a specified protein activity by screening for a specified protein activity in a library of clones prepd. by (i) recovering DNA from a DNA population derived from at least one uncultivated microorganism; and (ii) transforming a host with recovered DNA to produce a library of clones which is screened for the specified protein activity. Procedures used to generate a gene library from a sample of the exterior surface of a whale bone found at 1240 m depth in the Santa Catalina Basin are presented. A procedure for screening the expression library for hydrolase activity and to further characterize the type of hydrolase activity is also presented.

L163 ANSWER 5 OF 11 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:793762 CAPLUS
DOCUMENT NUMBER: 137:305698
TITLE: Methods for the preparation of nucleic acid librairies using gene shuffling for the identification mutants with desired characteristics
INVENTOR(S): **Delagrave, Simon**
PATENT ASSIGNEE(S): Hercules Incorporated, USA

Searched by Barb O'Bryen, STIC 308-4291

SOURCE: PCT Int. Appl., 46 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002081643	A2	20021017	WO 2002-US10905	20020404

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 2001-281587P P 20010405
US 2002-114379 A 20020402

AB Methods of directed fragmentation of polynucleotides combined with fragment interchange and ligation are provided for the prepn. of polynucleotide libraries. Fragmentation can be facilitated by at least one oligonucleotide adapter capable of directing polynucleotide cleavage at homologous sites among a set of parent polynucleotides. Libraries generated by the above methods can be screened for polynucleotides with desired characteristics or properties. Methods for the synthesis of polynucleotides and derivs. thereof are provided. Methods for the prepn. of combinatorial libraries of polynucleotides are also provided. In particular, methods for prepg. large polynucleotides of arbitrary sequence and in a manner that will readily lend itself to automation are provided. The present invention provides methods of prepg. a polynucleotide having at least 200 nucleotides in either a 5' to 3' or 3' to 5' direction by ligating a plurality of oligonucleotides, the assembly of which, represents the nucleotide sequence of the desired polynucleotide. The present invention also provides libraries of polynucleotides and screening of libraries for polynucleotide members having desired properties. The method is exemplified by shuffling of galactose oxidase (GO) mutants using defined oligonucleotide adapters, or random oligonucleotide adapters, or defined sequence adapter oligonucleotides, or native class IIS restriction site adapter oligonucleotide. Shuffling of flavivirus RNA genomes using yellow fever 17D vaccine strain is also performed.

L163 ANSWER 6 OF 11 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:754630 CAPLUS
DOCUMENT NUMBER: 137:274031
TITLE: Methods for the enzymatic assembly of polynucleotides and identification of polynucleotides having desired characteristics
INVENTOR(S): **Delagrave, Simon; Marrs, Barry**
PATENT ASSIGNEE(S): Hercules Incorporated, USA
SOURCE: PCT Int. Appl., 132 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002077289	A1	20021003	WO 2002-US8816	20020320

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,

CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 2001-813408 A 20010321

AB Methods for the synthesis of polynucleotides and derivs. thereof are provided. Methods for the prepn. of combinatorial libraries of polynucleotides are also provided. In particular, methods for prepg. large polynucleotides of arbitrary sequence and in a manner that will readily lend itself to automation are provided. The present invention provides methods of prepg. a polynucleotide having at least 200 nucleotides in either a 5' to 3' or 3' to 5' direction by ligating a plurality of oligonucleotides, the assembly of which, represents the nucleotide sequence of the desired polynucleotide. The present invention also provides libraries of polynucleotides and screening of libraries for polynucleotide members having desired properties.

REFERENCE COUNT: 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L163 ANSWER 7 OF 11 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:202397 CAPLUS
DOCUMENT NUMBER: 126:209724
TITLE: Altered bacteriochlorophyll and carotenoid binding in combinatorial LH2 mutants of Rhodobacter capsulatus
AUTHOR(S): Hu, Qinghui; **Delagrave, Simon**; Youvan, Douglas C.; Niederman, Robert A.
CORPORATE SOURCE: Department of Molecular Biology and Biochemistry, Rutgers University, Piscataway, NJ, 08855-1059, USA
SOURCE: Photosynthesis: From Light to Biosphere, Proceedings of the International Photosynthesis Congress, 10th, Montpellier, Fr., Aug. 20-25, 1995 (1995), Volume 1, 211-214. Editor(s): Mathis, Paul. Kluwer: Dordrecht, Neth.
CODEN: 64DFAW
DOCUMENT TYPE: Conference
LANGUAGE: English

AB The stability and low temp. optical properties of the light harvesting complex II (LH 2) formed by several combinatorial mutants with residue changes in the C-terminal half of the .beta.-polypeptide are described in this paper. Many of their phenotypic characteristics can be explained by the at.-resoln. structure detd. recently for the LH 2 complex of Rhodopseudomonas acidophila by X-ray crystallog., thereby illustrating the utility of combinatorial mutagenesis approaches for revealing crucial structure-function relationship. Pigment content and 77 K near-IR absorption maxima of mutant chromatophores were presented.

L163 ANSWER 8 OF 11 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1994:98425 CAPLUS
DOCUMENT NUMBER: 120:98425
TITLE: Searching sequence space to engineer proteins: exponential ensemble mutagenesis
AUTHOR(S): **Delagrave, Simon**; Youvan, Douglas C.
CORPORATE SOURCE: Dep. Chem., Massachusetts Inst. Technol., Cambridge, MA, 02139, USA
SOURCE: Bio/Technology (1993), 11(13), 1548-52
CODEN: BTCHDA; ISSN: 0733-222X
DOCUMENT TYPE: Journal
LANGUAGE: English

AB An efficient method for generating combinatorial libraries with a high

percentage of unique and functional mutants is described. Combinatorial libraries have been successfully used in the past to express ensembles of mutant proteins in which all possible amino acids are encoded at a few positions in the sequence. However, as more positions are mutagenized the proportion of functional mutants is expected to decrease exponentially. Small groups of residues were randomized in parallel to identify, at each altered position, amino acids which lead to functional proteins. By using optimized nucleotide mixts. deduced from the sequences selected from the random libraries, 16 sites in a model pigment-binding protein were simultaneously altered; .apprx.1% of the obsd. mutants were functional. Math. formalization and extrapolation of these exptl. data suggests that a 107-fold increase in the throughput of functional mutants was obtained relative to the expected frequency from a random combinatorial library. Exponential ensemble mutagenesis should be advantageous in cases where many residues must be changed simultaneously to achieve a specific engineering goal, as in the combinatorial mutagenesis of phage displayed antibodies. With the enhanced functional mutant frequencies obtained by this method, entire proteins could be mutagenized combinatorially.

L163 ANSWER 9 OF 11 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-09691 BIOTECHDS
TITLE: Synthesis of large polynucleotides, comprising synthesis of smaller oligonucleotides which are ligated together to form the polynucleotide is useful to provide gene libraries which can be screened for desired properties;
DNA array production by phage T4 RNA-ligase, ribozyme, nucleoside-oxidase, subtilisin, terminal-transferase, phosphatase and asymmetric polymerase chain reaction
AUTHOR: **DELAGRAVE S; MARRS B**
PATENT ASSIGNEE: HERCULES INC
PATENT INFO: WO 2001088173 22 Nov 2001
APPLICATION INFO: WO 2000-US14966 16 May 2000
PRIORITY INFO: US 2000-571774 16 May 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-171372 [22]
AB DERWENT ABSTRACT:
NOVELTY - Preparing a polynucleotide of at least 200 nucleotides, comprising providing oligonucleotides which make up the polynucleotide and ligating them together, is new.
DETAILED DESCRIPTION - Preparing a polynucleotide of at least 200 nucleotides and a predetermined sequence comprising: (a) providing a solid support; (b) providing a number of oligonucleotides whose combination of sequences comprises the sequence of the polynucleotide; (c) contacting the solid support with the 3' terminus of a first oligonucleotide to provide a tethered oligonucleotide; (d) ligating the 3' end of another oligonucleotide to the 5' terminus of the first oligonucleotide; and (e) repeating step (d) until the polynucleotide is prepared. Alternatively in (c) the 5' end is tethered and in step (d) the 5' end of another oligonucleotide is ligated to the 3' end of the tethered oligonucleotide. INDEPENDENT CLAIMS are included for the following: (i) preparing a polynucleotide from a number of oligonucleotides, comprising: (a) blocking the 3' terminus of a first oligonucleotide which comprises the 3' terminus of the polynucleotide; (b) coupling the 3' terminus of a further oligonucleotide to the 5' terminus of the blocked oligonucleotide; (c) amplifying the coupled oligonucleotide to form an amplified oligonucleotide substantially free of blocking group; and (d) repeating steps (a) to (c) with the amplified oligonucleotide until the polynucleotide is prepared; (ii) preparing a polynucleotide from a number of oligonucleotides, comprising: (a) blocking the 3' terminus of each of the oligonucleotides except the one that comprises the 5' terminus of the polynucleotide; (b) coupling the 3' terminus of the unblocked oligonucleotide with one of the blocked

oligonucleotides; (c) amplifying the coupled oligonucleotide to form an amplified oligonucleotide substantially free of blocking groups; and (d) repeating steps (b) and (c) with the amplified oligonucleotide until the polynucleotide is prepared; (iii) preparing library of polynucleotides, comprising simultaneously generating a number of different polynucleotides by coupling a number of oligonucleotides using a ligase, where at least one of the oligonucleotides is attached to a solid support; (iv) preparing a library of polynucleotides comprising simultaneously generating a number of different polynucleotides using the method of (i) or (ii); (v) a library of polynucleotides prepared by any of the claimed methods; and (vi) identifying a polynucleotide with a property, comprising generating a library using one of the claimed methods and selecting a polynucleotide with the desired property.

BIOTECHNOLOGY - Preferred Methods: The solid support is agarose, polyacrylamide, magnetic beads, polystyrene, polyacrylate, controlled pore glass, hydroxyethylmethacrylate, polyamide, polyethylene, polyethylenoxy or a polyethylenoxy/polystyrene copolymer. Ligation is carried out with a RNA ligase or ribozyme, preferably RNA ligase or modified or unmodified T4 RNA ligase. In the method of the main claim, if the 3' terminus is tethered, the first oligonucleotide is phosphorylated prior to or after contact with the solid support, preferably with a phosphoramidite or kinase. If the 3' terminus is tethered, unphosphorylated 5' terminus is capped with an enzyme that oxidizes the 5'-OH, preferably a nucleoside oxidase, or acylation using a lipase or subtilisin. The other oligonucleotides are 5' phosphorylated after ligation by forming a phosphamide or by reaction with a blocking oligonucleotide, preferably a 5'deoxyoligonucleotide or an oligonucleotide comprising a 5' fluorescent label. If the 5' terminus is tethered, the 3' termini of the other oligonucleotides are blocked or capped, preferably by phosphorylation or using enzymes which acylate the 3' terminus, more preferably a lipase or subtilisin or an enzyme which adds at least one dideoxy nucleotide, preferably terminal transferase. After ligation the 3' terminus is unblocked using a phosphatase, subtilisin or lipase. In the method of additional claims (i) and (ii) the blocking group comprising a solid support as defined above or ddUTP-biotin.. Coupling comprises contacting the blocked oligonucleotide with ligase and cosubstrate to form activated oligonucleotide, washing the activated oligonucleotide with contacting it with the further oligonucleotide and ligase. Amplification is by asymmetric PCR. In method (ii) the oligonucleotides are hybridized to bridging oligonucleotides before coupling and coupling is carried out sequentially. The polynucleotide is a gene, plasmid, viroid, or polynucleotide comprising an origin of replication. Preferably a number of polynucleotides are prepared simultaneously, preferably degenerate polynucleotides.

USE - The invention is used to generate gene libraries which can be used to screen for genes with desired properties which may allow discovery or development of new and improved biomolecules such as enzymes with increased activity or receptors with higher ligand affinity.

EXAMPLE - 20 oligonucleotides of 50 nucleotides are synthesized deprotected and purified by HPLC using standard techniques. Oligonucleotides are numbered according to their position in the polynucleotide with the 3' most being number 1. A linker containing a primary amine is attached to the 3'-OH of oligo1 so that its functional group is free to react with a solid support. Oligo1 is immobilized onto the support which may be magnetic beads according to the manufacturers instructions. Unbound oligonucleotides are washed off using a conventional solution. The attached oligo1 is phosphorylated at the 5'-OH group using standard technique and washed, if not already phosphorylated. Oligo2 is incubated with the oligo1 and ligase under conditions appropriate to the particular ligase. The nascent polynucleotides and fortuitously hybridized oligonucleotides are denatured and the nascent polynucleotide phosphorylated and washed. The nascent polynucleotide is then incubated with oligo3 and so on until all oligonucleotides are

assembled. The linker is removed to separate the polynucleotide from the solid support, and the polynucleotide is amplified using standard PCR. (29 pages)

L163 ANSWER 10 OF 11 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 2001-648219 [74] WPIDS
DOC. NO. CPI: C2001-191208
TITLE: Novel variant galactose oxidase enzyme for oxidizing a compound such as guar which is useful in paper making, for enzymatic synthesis of aldehydes and pulp biobleaching, has at least one substituted amino acid.
DERWENT CLASS: B04 D16 F09
INVENTOR(S): BYLINA, E J; COLEMAN, W J; **DELAGRAVE, S**; MAFFIA, A M; MURPHY, D J; RITTENHOUSE PRUSS, J L; BYLINA, E; RITTENHOUSE PRUSS, J
PATENT ASSIGNEE(S): (HERC) HERCULES INC; (BYLI-I) BYLINA E J; (COLE-I) COLEMAN W J; (DELA-I) DELAGRAVE S; (MAFF-I) MAFFIA A M; (MURP-I) MURPHY D J; (PRUS-I) RITTENHOUSE PRUSS J L
COUNTRY COUNT: 94
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2001062938	A2	20010830	(200174)*	EN	65
AU 2001041674	A	20010903	(200202)		
US 2001051369	A1	20011213	(200204)		

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2001062938	A2	WO 2001-US5732	20010221
AU 2001041674	A	AU 2001-41674	20010221
US 2001051369	A1 Provisional	US 2000-185001P	20000225
		US 2001-782906	20010214

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2001041674	A Based on	WO 200162938

PRIORITY APPLN. INFO: US 2001-782906 20010214; US 2000-185001P 20000225

AB WO 200162938 A UPAB: 20011217
NOVELTY - A variant galactose oxidase (vGO) protein (I) which differs from a wildtype galactose oxidase protein by at least one substituted amino acid, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a mutant polynucleotide (mgo) (II) which encodes (I) with superior enzymatic activity, which differs from a wildtype galactose oxidase-encoding polynucleotide by at least one nondegenerate codon; (2) a vector (III) comprising (II); (3) a cell comprising (III); and (4) an isolated antibody (IV) which is specific for an epitope of (I).

USE - (I) is useful for oxidizing a compound comprising a hydroxyl group, such as guar, methyl- alpha -D-galactose, galactose, lactose, raffinose, diethylene glycol, ethanol and other primary alcohols, guaran

guns, D-galacto-hexodialdose, dihydroxyacetone, 3-hydroxy-2-oxo-propionaldehyde, glycerol, S(-)-glyceraldehyde, 6-carboxyraffinose, methyl- alpha -D-galactopyranose, methyl- beta -D-galactopyranose, major glycolipid of human red cells, D-talose, 3-halo-1,2-propane-diols, GM1 ganglioside, D-galactosamine, melibiose, stachyose, desialyated glycoproteins (e.g. fetuin, mucin), N-acetyl-D-galactosamine, isopropyl-beta -D-thiogalactosylpyranoside, beta -thiodigalactoside, melibiitol, melibionic acid, 1,5-anhydrogalactitol, planteose, 2-glycerol- alpha -D-galactopyranoside, galactobiose, D-glucose, beta -D-galactopyranosyl and methyl- beta -D-thiogalactosylpyranoside. (I) is also useful for making paper by contacting pulp with a guar which has been oxidized by (I) (claimed). (I) is also useful in generation of H2O2 in situ, enzymatic synthesis of other aldehydes, pulp biobleaching, the use of GO-Schiff's reagent for early detection and prognosis in human colorectal adenocarcinoma and use of GO-glucan binding domain fusion proteins as targeting inhibitors of dental plaque bacteria. (I) is useful as a research tool for identification, characterization and purification of interacting regulatory proteins. (II) is useful for large-scale expression of (I) and also permits identification and isolation of polynucleotides encoding (I), such as human allelic variants and species homologs. The polynucleotides are also useful in hybridization assays to detect the capacity of cells to express (I) and fragments of (II) are useful as probes for detecting full-length or fragment mgo polynucleotides. (IV) is useful for detecting or quantifying vGO and purifying vGO.

ADVANTAGE - (I) has an enzymatic activity at least 1.35 fold greater than the wildtype protein, which is measured by Vmax/Km greater than 0.005 and improved thermostability (claimed). (I) is superior to wildtype galactose oxidase in terms of efficiency of oxidizing guar and other components.

Dwg.0/0

L163 ANSWER 11 OF 11 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 2002-129474 [17] WPIDS
DOC. NO. NON-CPI: N2002-097619
DOC. NO. CPI: C2002-039610
TITLE: Separating organic compounds from a plant root by immersing the root system in a solution of lyases and hydrolases to break down the mucilaginous sheath is non-destructive to the plant and provides compounds for pharmaceutical research.
DERWENT CLASS: B04 C06 D16 P11 P13
INVENTOR(S): **MARRS, B L**
PATENT ASSIGNEE(S): (PHAR-N) PHARMALEADS INC
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
US 6324785	B1	20011204	(200217)*		2

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
US 6324785	B1	US 1999-351244	19990712

PRIORITY APPLN. INFO: US 1999-351244 19990712
AB US 6324785 B UPAB: 20020313

NOVELTY - Separating organic compounds from the root of an intact plant, comprising immersing the root system with the mucilaginous sheath substantially intact, into an aqueous medium containing enzymes which break down the sheath so that organic compounds within the sheath are

released into the aqueous medium, is new.

USE - The method is used to extract from plant roots organic compounds that provide lead compounds for the pharmaceutical research industry

ADVANTAGE - Unlike prior art, the plant is not destroyed during the extraction process.

Dwg.0/0

```
=> fil capl
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(2)


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FILE COVERS 1907 - 27 Dec 2002  VOL 138 ISS 1
FILE LAST UPDATED: 26 Dec 2002  (20021226/ED)

  This file contains CAS Registry Numbers for easy and accurate
  substance identification.

CAS roles have been modified effective December 16, 2001. Please
check your SDI profiles to see if they need to be revised. For
information on CAS roles, enter HELP ROLES at an arrow prompt or use
the CAS Roles thesaurus (/RL field) in this file.

=> d que l12; d que l17; d que l21; d que l24; d que l27

L4         17399 SEA FILE=CAPLUS ABB=ON  LIBRAR?/OBI
L7         24949 SEA FILE=CAPLUS ABB=ON  3(1W)(END# OR TERMIN?)
L8         21724 SEA FILE=CAPLUS ABB=ON  5(1W)(END# OR TERMIN?)
L9        471705 SEA FILE=CAPLUS ABB=ON  BLOCK?
L10          205 SEA FILE=CAPLUS ABB=ON  L7(5A)L9
L11           49 SEA FILE=CAPLUS ABB=ON  L10 AND L8
L12            1 SEA FILE=CAPLUS ABB=ON  L4 AND L11



L4         17399 SEA FILE=CAPLUS ABB=ON  LIBRAR?/OBI
L9        471705 SEA FILE=CAPLUS ABB=ON  BLOCK?
L13        59979 SEA FILE=CAPLUS ABB=ON  OLIGONUCLEOTIDE#
L14        16723 SEA FILE=CAPLUS ABB=ON  POLYNUCLEOTIDE#
L15          741 SEA FILE=CAPLUS ABB=ON  L9(5A)L13
L17            2 SEA FILE=CAPLUS ABB=ON  L15 AND L4 AND L14



L4         17399 SEA FILE=CAPLUS ABB=ON  LIBRAR?/OBI
L9        471705 SEA FILE=CAPLUS ABB=ON  BLOCK?
L13        59979 SEA FILE=CAPLUS ABB=ON  OLIGONUCLEOTIDE#
L15          741 SEA FILE=CAPLUS ABB=ON  L9(5A)L13
L19        315593 SEA FILE=CAPLUS ABB=ON  TARGET?
L21             4 SEA FILE=CAPLUS ABB=ON  L15 AND L4 AND L19



L4         17399 SEA FILE=CAPLUS ABB=ON  LIBRAR?/OBI
L9        471705 SEA FILE=CAPLUS ABB=ON  BLOCK?
L13        59979 SEA FILE=CAPLUS ABB=ON  OLIGONUCLEOTIDE#
L14        16723 SEA FILE=CAPLUS ABB=ON  POLYNUCLEOTIDE#
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L24           5 SEA FILE=CAPLUS ABB=ON  L4 AND L13 AND L14 AND L9


L4        17399 SEA FILE=CAPLUS ABB=ON  LIBRAR?/OBI
L7        24949 SEA FILE=CAPLUS ABB=ON  3(1W)(END# OR TERMIN?)
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L13       59979 SEA FILE=CAPLUS ABB=ON  OLIGONUCLEOTIDE#
L14       16723 SEA FILE=CAPLUS ABB=ON  POLYNUCLEOTIDE#
L22      608867 SEA FILE=CAPLUS ABB=ON  COUPL?
L23      818057 SEA FILE=CAPLUS ABB=ON  EXTEN?
L27           7 SEA FILE=CAPLUS ABB=ON  L13 AND L14 AND L22 AND L23 AND (L9 OR
                L4 OR L7 OR L8)


=> s (l12 or l17 or l21 or l24 or l27) not l162

L164         14 (L12 OR L17 OR L21 OR L24 OR L27) NOT L162

=> fil wpids; d que l61; d que l64

FILE 'WPIDS' ENTERED AT 16:29:59 ON 27 DEC 2002
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FILE LAST UPDATED:          20 DEC 2002      <20021220/UP>
MOST RECENT DERWENT UPDATE:     200282        <200282/DW>
DERWENT WORLD PATENTS INDEX SUBSCRIBER FILE, COVERS 1963 TO DATE

>>> PATENT IMAGES AVAILABLE FOR PRINT AND DISPLAY >>>

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    SEE http://www.derwent.com/dwpi/updates/dwpicov/index.html <<<

>>> FOR A COPY OF THE DERWENT WORLD PATENTS INDEX STN USER GUIDE,
    PLEASE VISIT:
 http://www.stn-international.de/training_center/patents/stn_guide.pdf <<<

>>> FOR INFORMATION ON ALL DERWENT WORLD PATENTS INDEX USER
    GUIDES, PLEASE VISIT:
    http://www.derwent.com/userguides/dwpi_guide.html <<<


L43       10779 SEA FILE=WPIDS ABB=ON  OLIGONUCLEOTIDE# OR OLIGO NUCLEOTIDE#
L44       15218 SEA FILE=WPIDS ABB=ON  POLY NUCLEOTIDE# OR POLYNUCLEOTIDE#
L45      690665 SEA FILE=WPIDS ABB=ON  BLOCK?
L46       10343 SEA FILE=WPIDS ABB=ON  3(1W)(END# OR TERMIN?)
L47        8048 SEA FILE=WPIDS ABB=ON  5(1W)(END# OR TERMIN?)
L56       12711 SEA FILE=WPIDS ABB=ON  LIBRAR?
L60         314 SEA FILE=WPIDS ABB=ON  (L43 OR L46)(5A)L45
L61           2 SEA FILE=WPIDS ABB=ON  L44 AND L56 AND L46 AND L47 AND L60


L43       10779 SEA FILE=WPIDS ABB=ON  OLIGONUCLEOTIDE# OR OLIGO NUCLEOTIDE#
L44       15218 SEA FILE=WPIDS ABB=ON  POLY NUCLEOTIDE# OR POLYNUCLEOTIDE#
L48      660685 SEA FILE=WPIDS ABB=ON  COUPL?
L49      898763 SEA FILE=WPIDS ABB=ON  EXTEN?
L56       12711 SEA FILE=WPIDS ABB=ON  LIBRAR?
L64           4 SEA FILE=WPIDS ABB=ON  L43 AND L44 AND L48 AND L49 AND L56


=> s (l61 or l64) not l42
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previously printed w/ inventor search

L165 5 (L61 OR L64) NOT L42 previously printed

=> fil biosis; d que l82; d que l97

FILE 'BIOSIS' ENTERED AT 16:30:02 ON 27 DEC 2002
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FILE COVERS 1969 TO DATE.
CAS REGISTRY NUMBERS AND CHEMICAL NAMES (CNs) PRESENT
FROM JANUARY 1969 TO DATE.

RECORDS LAST ADDED: 25 December 2002 (20021225/ED)

L70	41735	SEA FILE=BIOSIS ABB=ON	OLIGONUCLEOTIDE# OR OLIGO NUCLEOTIDE#
L71	7751	SEA FILE=BIOSIS ABB=ON	POLY NUCLEOTIDE# OR POLYNUCLEOTIDE#
L72	16856	SEA FILE=BIOSIS ABB=ON	3(1W)(END# OR TERMIN?)
L76	328950	SEA FILE=BIOSIS ABB=ON	BLOCK?
L79	571	SEA FILE=BIOSIS ABB=ON	(L72 OR L70)(5A)L76
L81	12	SEA FILE=BIOSIS ABB=ON	L79 AND L71
L82	2	SEA FILE=BIOSIS ABB=ON	L81 AND (SOLID PHASE OR PROTECTION)

L68	44584	SEA FILE=BIOSIS ABB=ON	LIBRAR?
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L72	16856	SEA FILE=BIOSIS ABB=ON	3(1W)(END# OR TERMIN?)
L73	15585	SEA FILE=BIOSIS ABB=ON	5(1W)(END# OR TERMIN?)
L74	29	SEA FILE=BIOSIS ABB=ON	DDUTP
L75	163	SEA FILE=BIOSIS ABB=ON	T4(3A)(RNA OR RIBONUCLEIC OR RIBO NUCLEIC)(3A)LIGASE#
L76	328950	SEA FILE=BIOSIS ABB=ON	BLOCK?
L77	142283	SEA FILE=BIOSIS ABB=ON	COUPL?
L78	484664	SEA FILE=BIOSIS ABB=ON	EXTEN?
L83	61	SEA FILE=BIOSIS ABB=ON	L71 AND L68
L95	216612	SEA FILE=BIOSIS ABB=ON	TARGET?
L97	2	SEA FILE=BIOSIS ABB=ON	L83 AND L95 AND (L70 OR (L72 OR L73 OR L74 OR L75 OR L76 OR L77 OR L78))

=> s (l82 or l97) not l67

L166 4 (L82 OR L97) NOT L67 previously printed

=> fil biotechno; d que l117;d que l127

FILE 'BIOTECHNO' ENTERED AT 16:30:03 ON 27 DEC 2002
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FILE LAST UPDATED: 17 DEC 2002 <20021217/UP>
FILE COVERS 1980 TO DATE.

>>> SIMULTANEOUS LEFT AND RIGHT TRUNCATION AVAILABLE IN /CT AND BASIC INDEX <<<

L102	7905	SEA FILE=BIOTECHNO ABB=ON	OLIGONUCLEOTIDE/CT
L103	1462	SEA FILE=BIOTECHNO ABB=ON	POLYNUCLEOTIDE#
L104	10999	SEA FILE=BIOTECHNO ABB=ON	3(1W)(END# OR TERMIN?)
L108	128008	SEA FILE=BIOTECHNO ABB=ON	BLOCK? OR CAP####

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L113      29875 SEA FILE=BIOTECHNO ABB=ON  OLIGONUCLEOTIDE#
L114        720 SEA FILE=BIOTECHNO ABB=ON  (L113 OR L104)(5A)L108
L116        278 SEA FILE=BIOTECHNO ABB=ON  OLIGORIBONUCLEOTIDE/CT
L117          3 SEA FILE=BIOTECHNO ABB=ON  (L102 OR L116) AND L103 AND L114


L102       7905 SEA FILE=BIOTECHNO ABB=ON  OLIGONUCLEOTIDE/CT
L103       1462 SEA FILE=BIOTECHNO ABB=ON  POLYNUCLEOTIDE#
L104      10999 SEA FILE=BIOTECHNO ABB=ON  3(1W)(END# OR TERMIN?)
L106         57 SEA FILE=BIOTECHNO ABB=ON  T4(3A)(RNA OR RIBONUCLEIC OR RIBO
                NUCLEIC)(3A)LIGASE#
L107         15 SEA FILE=BIOTECHNO ABB=ON  DDUTP
L108     128008 SEA FILE=BIOTECHNO ABB=ON  BLOCK? OR CAP####
L116        278 SEA FILE=BIOTECHNO ABB=ON  OLIGORIBONUCLEOTIDE/CT
L127          1 SEA FILE=BIOTECHNO ABB=ON  (L102 OR L116 OR L104) AND L108 AND
                L103 AND (L106 OR L107)

=> s (l117 or l127) not l100

L167          3 (L117 OR L127) NOT (L100)  previously printed

=> fil biotechds; d que l155; d que l157;d que l161

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FILE LAST UPDATED: 20 DEC 2002       <20021220/UP>

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L139       9007 SEA FILE=BIOTECHDS ABB=ON  LIBRAR?
L140       9591 SEA FILE=BIOTECHDS ABB=ON  POLYNUCLEOTIDE# OR POLY NUCLEOTIDE#

L141      12933 SEA FILE=BIOTECHDS ABB=ON  OLIGONUCLEOTIDE# OR OLIGO NUCLEOTIDE
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L143       3184 SEA FILE=BIOTECHDS ABB=ON  3(1W)(END# OR TERMIN?)
L147       6159 SEA FILE=BIOTECHDS ABB=ON  BLOCK? OR CAP OR CAPPED OR CAPPING
                OR CAPS
L154        188 SEA FILE=BIOTECHDS ABB=ON  (L143 OR L141)(5A)L147
L155          4 SEA FILE=BIOTECHDS ABB=ON  L140 AND L139 AND L154


L140       9591 SEA FILE=BIOTECHDS ABB=ON  POLYNUCLEOTIDE# OR POLY NUCLEOTIDE#

L141      12933 SEA FILE=BIOTECHDS ABB=ON  OLIGONUCLEOTIDE# OR OLIGO NUCLEOTIDE
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L142       9007 SEA FILE=BIOTECHDS ABB=ON  LIBRAR?
L143       3184 SEA FILE=BIOTECHDS ABB=ON  3(1W)(END# OR TERMIN?)
L144       2855 SEA FILE=BIOTECHDS ABB=ON  5(1W)(END# OR TERMIN?)
L147       6159 SEA FILE=BIOTECHDS ABB=ON  BLOCK? OR CAP OR CAPPED OR CAPPING
                OR CAPS
L157          3 SEA FILE=BIOTECHDS ABB=ON  L143 AND L144 AND L141 AND L140 AND
                L142 AND L147


L140       9591 SEA FILE=BIOTECHDS ABB=ON  POLYNUCLEOTIDE# OR POLY NUCLEOTIDE#
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L141      12933 SEA FILE=BIOTECHDS ABB=ON  OLIGONUCLEOTIDE# OR OLIGO NUCLEOTIDE
                #
L142       9007 SEA FILE=BIOTECHDS ABB=ON  LIBRAR?
L152        715 SEA FILE=BIOTECHDS ABB=ON  L140(5A)(PREP? OR SYNTHES? OR
                ASSEMB?)
L158       7068 SEA FILE=BIOTECHDS ABB=ON  COUPL?
L159      12018 SEA FILE=BIOTECHDS ABB=ON  EXTEN?
L161          5 SEA FILE=BIOTECHDS ABB=ON  L152 AND L141 AND L142 AND L158 AND
                L159


=> s (l155 or l157 or l161) not l138

L168          9 (L155 OR L157 OR L161) NOT L138 previously printed

=> dup rem l164,l166,l167,l165,l168
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PROCESSING COMPLETED FOR L164
PROCESSING COMPLETED FOR L166
PROCESSING COMPLETED FOR L167
PROCESSING COMPLETED FOR L165
PROCESSING COMPLETED FOR L168
L169         33 DUP REM L164 L166 L167 L165 L168 (2 DUPLICATES REMOVED)
                ANSWERS '1-14' FROM FILE CAPLUS
                ANSWERS '15-18' FROM FILE BIOSIS
                ANSWERS '19-20' FROM FILE BIOTECHNO
                ANSWERS '21-24' FROM FILE WPIDS
                ANSWERS '25-33' FROM FILE BIOTECHDS

=> d ibib ab 1-33

L169 ANSWER 1 OF 33  CAPLUS  COPYRIGHT 2002 ACS        DUPLICATE 1
ACCESSION NUMBER:         2001:152872  CAPLUS
DOCUMENT NUMBER:          134:203076
TITLE:                    Liquid array technology
INVENTOR(S):              Chandler, Mark B.
PATENT ASSIGNEE(S):       Luminex Corporation, USA
SOURCE:                   PCT Int. Appl., 62 pp.
                          CODEN: PIXXD2
DOCUMENT TYPE:            Patent
LANGUAGE:                 English
FAMILY ACC. NUM. COUNT:   1
PATENT INFORMATION:

     PATENT NO.        KIND  DATE          APPLICATION NO.  DATE
     ----------------  ----  --------      ---------------  --------
     WO 2001014589      A2   20010301      WO 2000-US22769  20000821
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WO 2001014589 A3 20020801
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
EP 1248853 A2 20021016 EP 2000-955728 20000821
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL
PRIORITY APPLN. INFO.: US 1999-149710P P 19990820
WO 2000-US22769 W 20000821

AB This invention is directed to compns. and methods of screening, sequencing, and/or quantitating a nucleic acid of interest by hybridizing that nucleic acid with a set of **oligonucleotide** probes, which are **coupled** to fluorescently addressable multicolored microparticles. These microparticles are provided as a liq. array that can be positioned in predetd. wells or reaction vessels of a microtiter plate. For sequencing purposes, each such liq. array preferably comprises every possible combination of sequences for a given length of a probe. Hybridization occurs by complementary recognition of the analyte of interest with a probe. Probe, target, and/or competing mol. are tagged with a reporter mol. so that upon hybridization, the changes in fluorescence signal parameters are recorded and analyzed.

L169 ANSWER 2 OF 33 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:595043 CAPLUS
DOCUMENT NUMBER: 137:136032
TITLE: Methods and capture probes for high-throughput multiplex analysis of nucleic acid expression and single nucleotide polymorphism detection in molecular diagnosis of diseases
INVENTOR(S): Hinkel, Christopher A.; Kimmerly, William J.; Yang, Li
PATENT ASSIGNEE(S): Syngenta Participations Ag, Switz.
SOURCE: PCT Int. Appl., 63 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002061121	A2	20020808	WO 2002-EP868	20020128

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
PRIORITY APPLN. INFO.: US 2001-264972P P 20010129
US 2001-266186P P 20010202
US 2001-295986P P 20010604

AB Methods are provided for the multiplex anal. of **polynucleotide** expression and single nucleotide polymorphism detection using capture probes **coupled** to uniquely identified particles. The methods provided are characterized by high flexibility and high throughput. The

method for detg. **polynucleotide** expression involves hybridizing a first **oligonucleotide** primer to the target **polynucleotide**. CDNA is synthesized by reverse transcription of said target **polynucleotide** using first **oligonucleotide** primer wherein the **5' end** of the cDNA sequence corresponds to first **oligonucleotide** primer and the **3' end** of cDNA contains at least one nucleotide that **extends** beyond the **5' end** of the target **polynucleotide** to provided a single-stranded **extension**. A second **oligonucleotide** primer is hybridized to the single-stranded **extension** of cDNA on target **polynucleotide**. The cDNA on target **polynucleotide** is **extended** using a second primer and subsequently amplified in the presence of a detectable label. The amplified cDNA is digested and hybridized to a capture probe (specific for target **polynucleotide**), **coupled** to a solid particle like a fluorescent microbead. In another embodiment, multiple capture probes hybridize to different locations of the same target **polynucleotide**. Flow cytometry is used to det. if the digested cDNA is hybridized to said capture probe, thereby identifying the target **polynucleotide**. Methods for detecting single nucleotide polymorphism involve hybridizing primers (which contain unique hybridization tags that identify the primer which is not complementary to the sequence contg. the SNP of interest) to single-stranded **polynucleotides** contg. SNPs. The said hybridized primers are **extended** by primer **extension** to generate a product that contains a hybridization tag and a detectable label. The **extension** products are hybridized to capture probes by hybridization tags, where the capture probe is **coupled** to a particle like a microbead to identify the SNPs.

L169 ANSWER 3 OF 33 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:241008 CAPLUS
DOCUMENT NUMBER: 136:289892
TITLE: High throughput screening of mRNAs for areas accessible to antisense oligonucleotides
INVENTOR(S): Liang, Zicai; Zhang, Hong-Yan; Wahlestedt, Claes
PATENT ASSIGNEE(S): Neuromics Inc., USA
SOURCE: PCT Int. Appl., 41 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002024950	A2	20020328	WO 2001-SE2054	20010925
WO 2002024950	A3	20021205		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
US 2002187482	A1	20021212	US 2001-961700	20010924
AU 2001092468	A5	20020402	AU 2001-92468	20010925

PRIORITY APPLN. INFO.: US 2000-235029P P 20000925
WO 2001-SE2054 W 20010925

AB This invention relates to methods for identifying regions of RNA mols. that are available for interaction with small mols., particularly regions

that can hybridize with oligonucleotides having complementary sequences. Identifying such regions is useful in the design of probes, anti-sense oligonucleotides and small mol. drugs. The method involves using a library of oligonucleotides with a randomized core sequence and const. flanking regions. The const. regions may be **blocked** with **blocking oligonucleotides** to prevent non-specific hybridization. Sequences are hybridized to immobilized mRNAs under conditions in which the mRNA maintains its folded conformation. Sequences hybridizing to the mRNA are then amplified by PCR using the const. regions. Sequencing of the amplification products identifies the exposed regions of the mRNA that may be **targeted**, e.g by antisense oligonucleotides. The method is demonstrated by anal. of the accessible regions of the rabbit .beta.-globin mRNA.

L169 ANSWER 4 OF 33 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:921845 CAPLUS
TITLE: DNA shuffling and expression of hybrid **polynucleotides**
INVENTOR(S): Short, Jay M.
PATENT ASSIGNEE(S): Diversa Corporation, USA
SOURCE: U.S., 32 pp., Cont.-in-part of U.S. 5,965,408. CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 31
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6489145	B1	20021203	US 1997-962504	19971031
US 5965408	A	19991012	US 1996-677112	19960709
CA 2259628	AA	19980115	CA 1997-2259628	19970709
CA 2308292	AA	19990514	CA 1998-2308292	19981023
WO 9923236	A1	19990514	WO 1998-US22596	19981023
W: AU, CA, JP				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
AU 9912765	A1	19990524	AU 1999-12765	19981023
EP 1027450	A1	20000816	EP 1998-956185	19981023
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2002505845	T2	20020226	JP 2000-519091	19981023
US 6171820	B1	20010109	US 1999-246178	19990204
US 6352842	B1	20020305	US 1999-276860	19990326
US 6440668	B1	20020827	US 1999-376727	19990817
US 6358709	B1	20020319	US 2000-522289	20000309
US 6361974	B1	20020326	US 2000-535754	20000327
US 2002086279	A1	20020704	US 2001-875412	20010606

PRIORITY APPLN. INFO.:

US 1996-677112	A2	19960709
US 1995-8311P	P	19951207
US 1995-8316P	P	19951207
US 1996-651568	A2	19960522
US 1996-760489	A2	19961205
US 1997-962504	A	19971031
US 1997-988224	A1	19971210
WO 1998-US22596	W	19981023
US 1998-185373	A1	19981103
US 1999-246178	A2	19990204
US 1999-267118	A2	19990309
US 1999-276860	A2	19990326
US 1999-332835	B2	19990614
US 2000-495052	A2	20000131
US 2000-498557	A2	20000204

US 2000-522289 A2 20000309

AB To generate hybrid **polynucleotides** encoding biol. active polypeptides with enhanced activities, a method for producing random **polynucleotides** from the first and second polypeptides which share at least one region of partial sequence homol. by recombination and reductive reassortment is disclosed. A random priming kit using a non-proofreading polymerase was used to generate **polynucleotides** by priming at random sites on template with varying no. of dimers prepd. by UV light. The resulting **polynucleotides** were selected, isolated, and amplified. The PCR conditions including denaturation renaturation, and incubation time were selected in generating shuffled **polynucleotides**. The above **polynucleotides** were cloned and introduced into host cells to produce hybrid polypeptides. A method for screening of hybrid polypeptides by their biol. activities was also described.

REFERENCE COUNT: 34 THERE ARE 34 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L169 ANSWER 5 OF 33 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:130617 CAPLUS
DOCUMENT NUMBER: 130:191863
TITLE: Methods of identifying biological agent compositions using segmented copolymers
INVENTOR(S): Kabanov, Alexander V.; Alakov, Valery Y.; Pietrzynski, Grzegorz Jerzy
PATENT ASSIGNEE(S): Supratek Pharma Inc., Can.
SOURCE: PCT Int. Appl., 81 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9908112	A1	19990218	WO 1998-US16300	19980805
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9888981	A1	19990301	AU 1998-88981	19980805
EP 1005651	A1	20000607	EP 1998-940788	19980805
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2001512831	T2	20010828	JP 2000-506529	19980805

PRIORITY APPLN. INFO.:
US 1997-55256P P 19970808
US 1998-47109 A 19980324
WO 1998-US16300 W 19980805

AB New methods of identifying biol. agent compns. involving (a) prepg. a plurality of segmented copolymers, the segmented copolymers differing in at least one of the following, (i) at least one of their segment lengths, (ii) chem. structure, (iii) copolymer architecture; (b) prepg. compns. of the segmented copolymers with a biol. agent; (c) testing at least one of the compns. of segmented copolymers with a biol. agent for biol. properties using a cell, animal, plant or other biol. model, or measurement of a chem. or phys. property in a test tube, or a theor. model; and (d) identifying the compns. with desired biol. properties. The invention is designed to reduce the time and cost for creating desired drug compds. which are not only immediately ready for clin. trials, but

also possess a no. of important characteristics increasing the probability of the ultimate success. Unlike combinatorial chem., the invention does not discover new drug structures or alter the desirable drug's characteristics, but instead provides optimal compns. of a desired drug, solving the drug's problems of soly., bioavailability, resistance to metabolic enzymes, toxicity, membrane transport, site specific delivery, etc.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L169 ANSWER 6 OF 33 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:368306 CAPLUS
DOCUMENT NUMBER: 131:165837
TITLE: Extender PCR: a method for the isolation of sequences regulating gene expression from genomic DNA
AUTHOR(S): Anon.
CORPORATE SOURCE: USA
SOURCE: BioTechniques (1999), 26(5), 804-806
CODEN: BTNQDO; ISSN: 0736-6205
PUBLISHER: Eaton Publishing Co.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A new polymerase chain reaction (PCR)-based method is described for "walking" into previously uncloned regions of genomic DNA that negates the need for synthesis of double-stranded vectorette linkers, **blocked oligonucleotide** adaptors, or genomic DNA library construction and screening. The method relies on both the ligation of a single-stranded oligonucleotide adaptor to restriction enzyme-digested genomic DNA and the blocking of nonspecific replication of the adaptor-complementary strand by incorporation of a dideoxynucleotide. It involves Taq DNA polymerase-catalyzed extension from internal gene-specific sites that generates the complementary strand of the ligated adaptor sequence, thus producing the primer annealing sites necessary for the amplification of the desired **target** sequences. Restriction enzymes generating 5' overhanging ends can be used with this technique. Extender PCR can be used to clone both upstream (5') and/or downstream (3') regulatory regions using antisense or sense internal gene-specific primers, resp. If used in conjunction with long-range PCR protocols, this technique can rapidly amplify fragments of several kilobase pairs in length, negating the need to construct and screen genomic DNA libraries. It is particularly useful for the isolation of promoter regions from information contained within expressed sequence tag (EST) databases. This technique was used to isolate promoter sequences from three different opsin genes of mantis shrimp, Gonodactylus oerstedii, and the 5'-untranslated region and initiation codon of an opiate receptor-like gene from Lymnaea stagnalis.

REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L169 ANSWER 7 OF 33 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:799077 CAPLUS
DOCUMENT NUMBER: 130:149283
TITLE: Construction of a directed hammerhead ribozyme **library**: towards the identification of optimal **target** sites for antisense-mediated gene inhibition
AUTHOR(S): Pierce, Michael L.; Ruffner, Duane E.
CORPORATE SOURCE: Department of Bioengineering, University of Utah, Salt Lake City, UT, 84108, USA
SOURCE: Nucleic Acids Research (1998), 26(22), 5093-5101
CODEN: NARHAD; ISSN: 0305-1048
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English

Searched by Barb O'Bryen, STIC 308-4291

AB Antisense-mediated gene inhibition uses short complementary DNA or RNA **oligonucleotides** to **block** expression of any mRNA of interest. A key parameter in the success or failure of an antisense therapy is the identification of a suitable **target** site on the chosen mRNA. Ultimately, the accessibility of the **target** to the antisense agent dets. **target** suitability. Since accessibility is a function of many complex factors, it is currently beyond our ability to predict. Consequently, identification of the most effective **target**(s) requires examn. of every site. Towards this goal, we describe a method to construct directed ribozyme libraries against any chosen mRNA. The library contains nearly equal amts. of ribozymes **targeting** every site on the chosen transcript and the library only contains ribozymes capable of binding to that transcript. Expression of the ribozyme library in cultured cells should allow identification of optimal **target** sites under natural conditions, subject to the complexities of a fully functional cell. Optimal **target** sites identified in this manner should be the most effective sites for therapeutic intervention.

REFERENCE COUNT: 50 THERE ARE 50 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L169 ANSWER 8 OF 33 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:128068 CAPLUS
DOCUMENT NUMBER: 126:199798
TITLE: Enzymic synthesis of **oligonucleotides** using repeated cycles of primer **extension** with **blocked** nucleotides in a one pot method without intermediate purification
INVENTOR(S): Hyman, Edward D.
PATENT ASSIGNEE(S): Hyman, Edward D., USA
SOURCE: U.S., 29 pp., Cont.-in-part of U.S. 5, 516, 664.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 5
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 5602000	A	19970211	US 1995-464778	19950623
US 5436143	A	19950725	US 1992-995791	19921223
US 5516664	A	19960514	US 1993-161224	19931202

PRIORITY APPLN. INFO.: US 1992-995791 19921223
US 1993-100671 19930730
US 1993-161224 19931202

AB Enzymic synthesis of **oligonucleotides** is performed by the steps of: (a) combining a primer and a **blocked** nucleotide in the presence of a chain **extending** enzyme to form a primer-**blocked** nucleotide product contg. the **blocked** nucleotide **coupled** to the primer at its **3'-end**; (b) removing the **blocking** group from the **3' end** of the primer-**blocked** nucleotide product; and (c) repeating the cycle of steps (a) and (b), using the primer-nucleotide product of step (b) as the primer for step (a) in the next cycle, for sufficient cycles to form the **oligonucleotide** product. Cycles may optionally include the step of converting any unreacted **blocked** nucleotide to an unreactive form which is substantially less active as a substrate for the chain **extending** enzyme. Cycles may also include the step of removing the **blocking** group from unreacted **blocked** nucleotide. This step is unnecessary, however, when the same nucleotide is added in two or more successive cycles. The synthetic cycles are preferably performed in a single vessel without intermediate purifn. of **oligonucleotide** product.

L169 ANSWER 9 OF 33 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:416762 CAPLUS
DOCUMENT NUMBER: 127:34471
TITLE: Template process for preparing combinatorial **libraries**
INVENTOR(S): Ermantraut, Jewgeni; Woelfl, Stefan; Saluz, Hans-Peter
PATENT ASSIGNEE(S): Hans-Knoell-Institut Fuer Naturstoff-Forschung E.V., Germany
SOURCE: Ger. Offen., 8 pp.
CODEN: GWXXBX
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
DE 19543232	A1	19970515	DE 1995-19543232	19951107

AB A process is described for making combinatorial libraries of **oligonucleotides**, **polynucleotides**, peptides or polysaccharides by solid-phase synthesis. In this process the protected first building **block** is linked to a solid support and is deblocked selectively by using a template coated with the deblocking agent. The selectively deblocked substrate is then treated with the next building **block**, selectively deblocked using a template, and treated with the next building **block**. Use of the template allows very small loci to be addressed selectively and at the end of the synthesis the position of each planned compd. on the support is known. A library of 64 trinucleotides was prepd. on hydroxyethoxyethoxypropylsiloxane on glass using 4 template steps to attach each of the nucleotides in the chain.

L169 ANSWER 10 OF 33 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:535031 CAPLUS
DOCUMENT NUMBER: 127:243702
TITLE: Magnetic bead capture of cDNAs from double-stranded plasmid cDNA **libraries**
AUTHOR(S): Shepard, Allan R.; Rae, James L.
CORPORATE SOURCE: Departments Physiology/Biophysics and Ophthalmology, Mayo Foundation, Rochester, MN, 55905, USA
SOURCE: Nucleic Acids Research (1997), 25(15), 3183-3185
CODEN: NARHAD; ISSN: 0305-1048
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB We have developed a cDNA library screening method which allows the simultaneous screening of >1012 double-stranded plasmid cDNA mols. with minimal a priori sequence knowledge. A biotinylated, gene-specific **oligonucleotide** probe along with abutting **'blocking'** oligos is hybridized to the plasmid cDNA library and the **target** plasmid retrieved with paramagnetic streptavidin beads and transformed into Escherichia coli. Multiple rounds of enrichment with a **target** plasmid represented at 0.002-0.0001% resulted in over one-third pos. clones. Our method will be useful for isolating even the rarest cDNAs starting from ESTs, isolated exons or homologous sequence information.

L169 ANSWER 11 OF 33 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1996:350546 CAPLUS
DOCUMENT NUMBER: 125:115086
TITLE: Enzymic synthesis of repeat regions of **oligonucleotides**

Searched by Barb O'Bryen, STIC 308-4291

INVENTOR(S): Hyman, Edward D.
PATENT ASSIGNEE(S): USA
SOURCE: U.S., 16 pp., Cont.-in-part of U.S. Ser. No. 100,671.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 5
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 5516664	A	19960514	US 1993-161224	19931202
US 5436143	A	19950725	US 1992-995791	19921223
WO 9414972	A1	19940707	WO 1993-US12456	19931221
W: AU, CA, JP, US, US, US				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
CA 2150670	AA	19940707	CA 1993-2150670	19931221
AU 9458737	A1	19940719	AU 1994-58737	19931221
EP 675963	A1	19951011	EP 1994-904879	19931221
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, NL, PT, SE				
JP 08505053	T2	19960604	JP 1993-515397	19931221
US 5629177	A	19970513	US 1994-259308	19940613
US 5514569	A	19960507	US 1995-376857	19950123
US 5602000	A	19970211	US 1995-464778	19950623

PRIORITY APPLN. INFO.:
US 1992-995791 19921223
US 1993-100671 19930730
US 1992-921223 19921223
US 1993-161224 19931202
WO 1993-US12456 19931221
US 1994-259308 19940613

AB Enzymic synthesis of a repeat region of an **oligonucleotide** may be performed by the steps of: (a) combining a primer and a **blocked** nucleotide in the presence of a chain **extending** enzyme whereby a primer-**blocked** nucleotide product is formed contg. the **blocked** nucleotide **coupled** to the primer at its **3'-end**; (b) removing the **blocking** group from the **3'-end** of the primer-**blocked** nucleotide product using a 3'-phosphatase enzyme substantially without removing the 3'-phosphate **blocking** group from unreacted 3'-phosphate-**blocked** nucleotide; and (c) repeating the cycle of steps (a) and (b), using the primer-nucleotide product of step (b) as the primer for step (a) in the next cycle, for sufficient cycles to form the **oligonucleotide** product. These cycles are performed in a single vessel without intermediate purifn. of **oligonucleotide** product. Also disclosed is a process for synthesizing an **oligonucleotide** having a defined sequence including at least one repeat region and one non-repeating region, wherein at least one non-repeating region is synthesized by reaction cycles using the steps of **extending** a primer with a 3'-**blocked** nucleotide, inactivating unreacted 3'-**blocked** nucleotide, and removing the **blocking** group from the **extended** primer. ApApCpApA and ApApCpApApdA were synthesized using the disclosed processes.

L169 ANSWER 12 OF 33 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1986:494127 CAPLUS
DOCUMENT NUMBER: 105:94127
TITLE: Large scale production of DNA probes
INVENTOR(S): Dattagupta, Nanibhushan; Rae, Peter; Crothers, Donald; Barnett, Thomas
PATENT ASSIGNEE(S): Molecular Diagnostics, Inc., USA
SOURCE: Eur. Pat. Appl., 13 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent

Searched by Barb O'Bryen, STIC 308-4291

LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 184056	A2	19860611	EP 1985-114561	19851116
EP 184056	A3	19870415		
EP 184056	B1	19900131		
R: AT, BE, CH, DE, FR, GB, IT, LI, NL, SE				
US 4734363	A	19880329	US 1984-675386	19841127
CA 1264452	A1	19900116	CA 1985-486641	19850711
AT 49977	E	19900215	AT 1985-114561	19851116
JP 61227785	A2	19861009	JP 1985-265160	19851127

PRIORITY APPLN. INFO.: US 1984-675386 19841127
EP 1985-114561 19851116

AB A method for prepg. nucleic acid sequences on a large scale without continually using cloning or plasmid vectors is described. The method involves (a) covalently **coupling** a DNA strand complementary to the strand to be synthesized to a solid support so that its **3'-end** is adjacent to the solid support; (b) hybridizing an **oligonucleotide** corresponding to the **5'-end** of the desired strand to the complementary **polynucleotide**; and (c) contacting the hybridized intermediate with a polymerase and nucleotides so that the **oligonucleotide** grows at its **3'-end** following the **polynucleotide** as template to produce the desired strand. The structure constituting the **polynucleotide** base-paired to the **extended oligonucleotide** is denature so as to release the **oligonucleotide** into soln. The solid support is sepd. from the soln. and recycled for future use. The method is useful for producing anal. and diagnostic DNA probes.

L169 ANSWER 13 OF 33 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1981:528770 CAPLUS
DOCUMENT NUMBER: 95:128770
TITLE: Site-specific modification of E. coli small ribosomal subunit with an arylazide derivative of a heptadeoxyribonucleotide complementary to the **3'-end** region of 16S RNA
AUTHOR(S): Skripkin, E. A.; Krynetskii, E. Yu.; Veiko, V. P.; Kopylov, A. M.; Bogdanov, A. A.; Shabarova, Z. A.
CORPORATE SOURCE: Chem. Dep., M. V. Lomonosov State Univ., Moscow, USSR
SOURCE: Bioorganicheskaya Khimiya (1981), 7(7), 1040-6
CODEN: BIKHD7; ISSN: 0132-3423
DOCUMENT TYPE: Journal
LANGUAGE: Russian

AB The nucleotide d(AGGAGGT), which is complementary to the **3'-end** of Escherichia coli 16 S rRNA, was prepd., phosphorylated with ATP-.gamma.-32P and phage T4 **polynucleotide** kinase, and **coupled** to p-azidobenzoic acid with ethylenediamine; the methodol. has been used before to prep. similar octa- and pentanucleotide affinity labels. Incubation of 70 S ribosomes with 1 mol reagent/5 mol ribosomes followed by UV irradn. gave exclusive labeling of the 30 S subunit. The **extent** of labeling was as high as with 30 S subunits alone. Dissocn. of subunits followed by electrophoresis in 1 and 2 dimensions and in the presence and absence of SDS, as well as tryptic peptide mapping, showed the principal product of modification to be protein S5.

L169 ANSWER 14 OF 33 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1968:46503 CAPLUS
DOCUMENT NUMBER: 68:46503
TITLE: Nucleases of yeast. II. Purification, properties, and specificity of an endonuclease from yeast

AUTHOR(S): Lee, Se Yong; Nakao, Yoshio; Bock, Robert M.
CORPORATE SOURCE: Univ. of Wisconsin, Madison, WI, USA
SOURCE: Biochim. Biophys. Acta (1968), 151(1), 126-36
CODEN: BBACAQ
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A nuclease was purified .apprx.30-fold from the supernatant fraction of a hybrid yeast (Saccharomyces fragilis .times. S. dobzhanskii) by salt fractionation, chromatog. on Sephadex G-200, and DEAE-cellulose chromatog. The optimum pH is 7.6 and Mg2+ is required for the full activity. The action of this nuclease on polyribonucleotides is exclusively endonucleolytic. The major products upon **extensive** digestion of homopolymers are di- and trinucleotides having **5'**-phosphomonoester **end** groups. The formation of mononucleotide is slight. Poly A and poly U are hydrolyzed first to a family of small **oligonucleotides** having **5'**-phosphomonoester **end** groups. The distribution of these **oligonucleotides** is not completely random and depends on the conformation of the substrate. Hydrolysis of shorter chains is much slower than longer chains. This endonuclease has no apparent specificity for a particular base residue in **polynucleotides**. This mode of hydrolysis **coupled** with its high stability make this enzyme a very useful reagent for the prepn. of **oligonucleotides** with **5'**-phosphate **ends** from either synthetic **polynucleotides** or natural RNA. This enzyme appears to be specific for **polynucleotides** having a random coil conformation. Double- and triple-stranded helical conformations are less susceptible to attack. Poly A, poly U and poly I are hydrolyzed faster than poly C, transfer RNA, or ribosomal RNA. The enzyme prepn. hydrolyzes denatured DNA at approx. the same rate as yeast ribosomal RNA. Highly polymd. native Escherichia coli DNA is almost inactive as substrate. 16 references.

L169 ANSWER 15 OF 33 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 2
ACCESSION NUMBER: 1995:386854 BIOSIS
DOCUMENT NUMBER: PREV199598401154
TITLE: **Solid-phase** synthesis of oligoribonucleotides using T4 RNA ligase and T4 **polynucleotide** kinase.
AUTHOR(S): Vratskikh, L. V. (1); Komarova, N. I.; Yamkovoy, V. I. (1)
CORPORATE SOURCE: (1) Dep. Natural Sciences, Novosibirsk State University, Pirogova 2, Novosibirsk 630090 Russia
SOURCE: Biochimie (Paris), (1995) Vol. 77, No. 4, pp. 227-232.
ISSN: 0300-9084.
DOCUMENT TYPE: Article
LANGUAGE: English

AB The concept of **solid-phase** synthesis of oligoribonucleotide using T4 RNA ligase and T4 **polynucleotide** kinase has been proposed and tested with model homo-oligoribonucleotide. The method consists of the immobilization of the first oligomer **block** at the **3'-terminus** on a solid support followed by a chain elongation in the 5'-direction with trinucleoside diphosphates using T4 RNA-ligase and phosphorylation using **polynucleotide** kinase. Hydrazides of Biogel P-300, Sepharose 4B and cellulose were tested as solid supports for immobilization of initial oligomers. The properties of supports were rated on reactivities of immobilized 5'-phosphorylated oligomers as phosphate donors in the **solid phase** reactions, hydrodynamical properties and capacity to eliminate donor molecules spontaneously during reactions. Hydrazide of Sepharose 4B appeared to be a more suitable support because of better hydrodynamic properties and highest reactivities of immobilized donors. Saturated concentrations of RNA ligase and **polynucleotide** kinase and optimal time of joining reaction were determined. In a model

experiment ApApA was twice attached to the immobilized hydrazide of Sepharose 4B donor (pA)-6P-ox. The yield of (Ap)-12 was 25%.

L169 ANSWER 16 OF 33 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:71744 BIOSIS
DOCUMENT NUMBER: PREV200200071744
TITLE: Random truncation and amplification of nucleic acid.
AUTHOR(S): Lietz, Eric
ASSIGNEE: Genopsys, Inc.
PATENT INFORMATION: US 6319694 November 20, 2001
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (Nov. 20, 2001) Vol. 1252, No. 3, pp. No Pagination. ftp://ftp.uspto.gov/pub/patdata/. e-file.
ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
AB A method is provided for producing a **library** of mutagenized **polynucleotides** from a **target** sequence comprising (a) taking a sample comprising: (i) a **target** sequence including a section to be mutagenized, (ii) a **library** of first primers where the first primers include a first fixed sequence and a first unknown sequence 3' to the first fixed sequence, the first unknown sequence varying within the **library** of first primers, and (iii) a **library** of second primers where the second primer include a second fixed sequence that differs from the first fixed sequence, and a second unknown sequence 3' to the second fixed sequence, the second unknown sequence varying within the **library** of second primers; (b) performing one or more cycles of primer **extension** amplification on the sample in the presence of at least one polymerase such that a member of the **library** of the first primers is **extended** relative to the **target** sequence; and (c) performing one or more additional cycles of primer **extension** amplification on the sample such that a member of the **library** of the second primers is **extended** relative to the first primer that was **extended** in step (b) to form the **library** of mutagenized **polynucleotides**.

L169 ANSWER 17 OF 33 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2001:462721 BIOSIS
DOCUMENT NUMBER: PREV200100462721
TITLE: Method and compositions for improved **polynucleotide** synthesis.
AUTHOR(S): Yang, Shuwei (1)
CORPORATE SOURCE: (1) Rockville, MD USA
ASSIGNEE: Genecopoeia, Inc., Frederick, MD, USA
PATENT INFORMATION: US 6274353 August 14, 2001
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (Aug. 14, 2001) Vol. 1249, No. 2, pp. No Pagination. e-file.
ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
AB The sensitivity and specificity of **polynucleotide** synthesis is increased by protecting the 3'-end of an oligonucleotide used as a primer in the synthesis of the **polynucleotide**. **Protection** of the 3'-end of an oligonucleotide prevents non-specific chain elongation. Removal of blocking group an elevated temperature, using a thermostable enzyme, permits template-specific **polynucleotide** synthesis. The present invention also provides oligonucleotides with a **3' end** protected by a **blocking** group and a thermostable enzyme capable of removing the blocking group at an elevated temperature. The compositions and methods of the invention are very useful in a variety of techniques for DNA/RNA amplification and analysis, including medical

genetics research and diagnosis, pathogen detection, forensic, and animal and plant genetics applications, among others.

L169 ANSWER 18 OF 33 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2000:308992 BIOSIS
DOCUMENT NUMBER: PREV200000308992
TITLE: Mapping of accessible sites for **oligonucleotide** hybridization on hepatitis delta virus ribozymes.
AUTHOR(S): Wrzesinski, Jan; Legiewicz, Michal; Ciesiolka, Jerzy (1)
CORPORATE SOURCE: (1) Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12/14, 61-704, Poznan Poland
SOURCE: Nucleic Acids Research, (April 15, 2000) Vol. 28, No. 8, pp. 1785-1793. print.
ISSN: 0305-1048.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Semi-random **libraries** of DNA 6mers and RNase H digestion were applied to search for sites accessible to hybridization on the genomic and antigenomic HDV ribozymes and their 3' truncated derivatives. An approach was proposed to correlate the cleavage sites and most likely sequences of oligomers, members of the **oligonucleotide libraries**, which were engaged in the formation of RNA-DNA hybrids. The predicted positions of oligomers hybridizing to the genomic ribozyme were compared with the fold of **polynucleotide** chain in the ribozyme crystal structure. The data exemplified the crucial role of **target** RNA structural features in the binding of antisense **oligonucleotides** . It turned out that cleavages were induced if the bound oligomer could adapt an ordered helical conformation even when it required partial penetration of an adjacent double-stranded region. The major features of RNA structure disfavoring hybridization and/or RNase H hydrolysis were sharp turns of the **polynucleotide** chain and breaks in stacking interactions of bases. Based on the predicted positions of oligomers hybridizing to the antigenomic ribozyme we chose and synthesized four antisense DNA 6mers which were shown to direct hydrolysis in the desired, earlier predicted regions of the molecule.

L169 ANSWER 19 OF 33 BIOTECHNO COPYRIGHT 2002 Elsevier Science B.V.
ACCESSION NUMBER: 1998:28179167 BIOTECHNO
TITLE: One-pot colorimetric differentiation of **polynucleotides** with single base imperfections using gold nanoparticle probes
AUTHOR: Storhoff J.J.; Elghanian R.; Mucic R.C.; Mirkin C.A.; Letsinger R.L.
CORPORATE SOURCE: C.A. Mirkin, Department of Chemistry, Northwestern University, 2145 Sheridan Road, Evanston, IL 60208, United States.
E-mail: CAMirkin@chem.nwu.edu
SOURCE: Journal of the American Chemical Society, (11 MAR 1998), 120/9 (1959-1964)
CODEN: JACSAT ISSN: 0002-7863
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Selective colorimetric **polynucleotide** detection based on Au nanoparticle probes which align in a 'tail-to-tail' fashion onto a target **polynucleotide** is described. In this new nanoparticle-based detection system, Au particles (.sim.13 nm diameter), which are **capped** with 3'- and 5'- (alkanethiol)**oligonucleotides**, are used to complex a 24-base **polynucleotide** target. Hybridization of the target with the probes results in the formation of an extended polymeric Au nanoparticle/**polynucleotide** aggregate,

which triggers a red to purple color change in solution. The color change is due to a red shift in the surface plasmon resonance of the Au nanoparticles. The aggregates exhibit characteristic, exceptionally sharp 'melting transitions' (monitored at 260 or 700 nm), which allows one to distinguish target sequences that contain one base end mismatches, deletions, or an insertion from the fully complementary target. When test solutions are spotted onto a C18 reverse-phase thin-layer chromatography plate, color differentiation is enhanced and a permanent record of the test is obtained, thereby providing a better method for distinguishing the aforementioned target sequences. Significantly, one-pot colorimetric detection of the target in the presence of four strands with single base imperfections can be accomplished with this new probe system.

L169 ANSWER 20 OF 33 BIOTECHNO COPYRIGHT 2002 Elsevier Science B.V.
ACCESSION NUMBER: 1993:23232233 BIOTECHNO
TITLE: Detection of single base substitutions in **polynucleotides** by **capture** with immobilized **oligonucleotides**
AUTHOR: Balaguer P.; Terouanne B.; Allibert P.; Cros P.; Boussioux A.-M.; Mandrand B.; Nicolas J.-C.
CORPORATE SOURCE: INSERM Unite 58, 60 Rue de Navacelles,34090 Montpellier, France.
SOURCE: Molecular and Cellular Probes, (1993), 7/2 (155-159) CODEN: MCPRE6 ISSN: 0890-8508
DOCUMENT TYPE: Journal; Article
COUNTRY: United Kingdom
LANGUAGE: English
SUMMARY LANGUAGE: English
AB We have improved a sandwich hybridization assay to detect single base substitutions in polymerase chain reaction (PCR) amplified DNA sequences. The target DNA was captured by an immobilized oligonucleotide and revealed using a second oligonucleotide coupled to an enzyme. Short oligonucleotides (13, 15 bases) were used to obtain specific hybridization at 37.degree.C. We developed two different assay formats for rapid identification of PCR products: a microtitration plate format with oligonucleotides bound to polystyrene and a channelling assay using oligonucleotides immobilized on Sepharose, which did not require any separation step. The specificity and advantages of both methods are described.

L169 ANSWER 21 OF 33 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 2002-750492 [81] WPIDS
DOC. NO. CPI: C2002-212671
TITLE: Identifying targets of effectors of gene expression or cellular activity, by contacting reporter cells with an effector, adding nucleic acid encoding a target and identifying cells with altered expression or activity.
DERWENT CLASS: B04 D16
INVENTOR(S): AZA-BLANC, P; CALDWELL, J S; CHANDA, S K; COOKE, M P; HOGENESCH, J B; SOMIA, N V
PATENT ASSIGNEE(S): (IRMI-N) IRM LLC
COUNTRY COUNT: 100
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2002072783	A2	20020919	(200281)*	EN	140

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VN YU ZA ZM

ZW

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2002072783	A2	WO 2002-US7713	20020312

PRIORITY APPLN. INFO: US 2001-275266P 20010312

AB WO 200272783 A UPAB: 20021216

NOVELTY - Identifying (M1) target of an effector (E) of gene expression (GE) or cellular activity (CA), comprises contacting an addressable collection (I) of reporter cells that generate an output representative of GE/CA, with (E), introducing nucleic acid (II) encoding potential target of (E) and identifying cells in (I) that exhibit expression or activity that is different in the presence of (II) than in its absence.

DETAILED DESCRIPTION - M1 Involves identifying the target of an effector or a target for an effector of GE or for CA, by providing (I), contacting the cells with an effector of GE or CA, introducing (II) (the contacting and introducing steps are performed either simultaneously or sequentially in either order), and identifying cells in (I) that exhibit expression or activity that is different in the presence of (II) than in its absence, therefore identifying the target of or for (E).

INDEPENDENT CLAIMS are also included for:

(a) identifying (M2) a function of endogenous gene by modulating the level of a product encoded by the endogenous gene, by introducing nucleic acid molecules into populations of RCs to form (I) (cells of a first cell population comprise a different introduced nucleic acid from cells of at least a second cell population), and identifying cell populations in the collection in which cells exhibit a phenotype that is different in the presence of (II) from the phenotype exhibited in its absence, therefore identifying a nucleic acid molecule that modulates the level of a product of an endogenous gene or genes that effect the phenotype and identifying the function of the endogenous gene or genes;

(b) identifying (M3) the targets of a perturbagen by modulating the level of an endogenous messenger RNA, by introducing (II) into populations of RCs to form (I), exposing the cells to a perturbagen that potentially alters a phenotype, and identifying cell populations in the collection in which cells exhibit a phenotype that is different in the presence of (II) and the perturbagen compared to the phenotype exhibited by the cells in the absence of (II) and the perturbagen;

(c) identifying a cDNA that, when expressed in a cell, causes an altered response of the cell to a biologically active molecule compared to a control cell;

(d) a database (I) produced by the above method;

(e) a combination (II) comprising (I), where RCs comprise a promoter operatively linked to a reporter gene, and a **library** of nucleic acid molecules; and

(f) a kit comprising (II), and optionally comprising any additional components selected from instructions for use of the kit for identifying targets of perturbations for GE or CA, and reagents for introducing (II) into cells.

USE - M1 Is useful for identifying a target of an effector or a target for effector for gene expression or for a cellular activity (claimed).

The method is useful to perform rational target selection, by altering concentrations of components of pathways and observing the phenotypic results to permit identification of rate limiting step(s) in a pathway. The methods are useful to identify the target as characterized perturbation, such as an effector or condition.

ADVANTAGE - The method is fully automated and provides an increased throughput over conventional methods. Miniaturization and automation of

transfection/transduction procedures permit comprehensive studies of phenotypes or pathways at the level of genome. The method combines the ability to measurably modulate the biological effect of a small molecule by overexpression of its target in cells, with the utility of laboratory automation and arrayed cDNA expression **library** formats to identify targets efficiently. As the process is automated, the speed is significantly increased and the cost is reduced.

DESCRIPTION OF DRAWING(S) - The figure shows the result of in cellulo competition experiments with HEK293:NF-kappaB reporter cells and Jurkat:NF-kappaB reporter cells.

Dwg.2/5

L169 ANSWER 22 OF 33 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 2002-188733 [24] WPIDS
CROSS REFERENCE: 2002-206219 [26]
DOC. NO. CPI: C2002-058394
TITLE: Producing nucleotide incorporating enzyme to incorporate rare nucleotide analog, by diversifying nucleic acids encoding parental enzyme to produce **library** encoding variants, detecting variant that incorporates analog.
DERWENT CLASS: B04 D16
INVENTOR(S): NESS, J; RAILLARD, S A; WELCH, M
PATENT ASSIGNEE(S): (MAXY-N) MAXYGEN INC; (NESS-I) NESS J; (RAIL-I) RAILLARD S A; (WELC-I) WELCH M
COUNTRY COUNT: 96
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2002010358	A2	20020207	(200224)*	EN	80

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW

AU 2001079134 A 20020213 (200238)
US 2002102577 A1 20020801 (200253)

APPLICATION DETAILS:

PATENT NO	KIND		APPLICATION	DATE
WO 2002010358	A2		WO 2001-US24181	20010731
AU 2001079134	A		AU 2001-79134	20010731
US 2002102577	A1	Provisional	US 2000-222056P	20000731
		Provisional	US 2000-244764P	20001031
			US 2001-920452	20010731

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 2001079134	A	Based on	WO 200210358

PRIORITY APPLN. INFO: US 2000-244764P 20001031; US 2000-222056P 20000731; US 2001-920452 20010731

AB WO 200210358 A UPAB: 20020820
NOVELTY - Producing nucleotide-incorporating enzyme (I) to incorporate non-natural/rare nucleotide analog (II), comprising providing nucleic acid segments (S) encoding parental (I), identifying (II) to be incorporated, diversifying (S) to produce a nucleic acid **library** encoding

variants of (I), and identifying variant that incorporates (II) at least 10 % or 10-fold as efficiently as natural **oligonucleotide**.

DETAILED DESCRIPTION - Producing (M1) nucleotide-incorporating enzyme (I) to incorporate non-natural/rare nucleotide analog (II), comprising:

(a) providing a number of nucleic acid segments (S) encoding all or part of one or more parental nucleotide incorporating enzymes (I) or its homolog;

(b) identifying at least one non-natural or rare nucleotide analog (II) to be incorporated by (I), where (II) is incorporated by (I) at an efficiency of less than 10 % the efficiency of a naturally occurring nucleotide (III);

(c) diversifying (S), to produce a **library** of nucleic acids encoding variants of (I); and

(d) identifying at least one variant of (I) that incorporates (II) at least 10 % or 10-fold as efficiently as (III).

Alternatively, producing (I) with increased tolerance to biological impurities, comprises:

(a) providing and diversifying (S), to produce a **library** of nucleic acids encoding variants of (I); and

(b) identifying at least one variant of (I) that efficiently polymerizes a **polynucleotide** in a template dependent manner in the presence of a biological impurity found in blood, plasma or urine.

INDEPENDENT CLAIMS are also included for the following:

(1) a nucleotide incorporating enzyme variant (I) produced by M1;

(2) a kit (K) comprising (I) and one or more container, a packaging material, and (II) or (III);

(3) an integrated system (II) comprising (I), (II) and a detector; and

(4) identifying (M2) nucleotide incorporating enzyme having a desired property.

USE - (I) is useful in polymerase chain reactions (to yield PCR products or to be able to disrupt secondary structure by elevated temperature stability during amplification reaction), sequencing reactions or other primary **extension** reactions in vitro. M2 is useful for identifying nucleotide incorporating enzymes with one or more desired properties, optionally **coupled** with the ability to efficiently incorporate a non-natural or rare nucleotide analog of interest.

ADVANTAGE - (II) is incorporated less efficiently than inosine by a parental (I), less efficiently than 7-deaza dGTP by a parental (I), or at an efficiency of less than 5 % the efficiency of (III) by a parental (I). Variant of (I) has thermostability, evenness of nucleotide incorporation, efficient terminal transferase activity, low or high fidelity, processivity, strand-displacement activity, nick translation activity, exchange reaction, cation requirement, modulation of activity by cation, sulfhydryl reagent requirement, shelf-life, salt tolerance, organic solvent tolerance, mechanical stress tolerance, tolerance to impurities, altered pH dependence, altered dependence on buffer conditions, template compositions, primer compositions and improved stability. (All claimed). Variant of (I) incorporates (II) at least 20 fold, preferably 100 fold, more efficiently than at least one parental (I). M1 is employed to produce nucleotide incorporating enzyme that incorporate a wide variety of different nucleotide analogs, regardless of the efficiency at which such a nucleotide analog is incorporated by existing enzymes. The variants demonstrate a significant increase in efficiency relative to a reference polymerase, such as the one or more parental polymerases from which the starting materials are derived.

Dwg.0/4

L169 ANSWER 23 OF 33 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 2002-114290 [15] WPIDS
DOC. NO. CPI: C2002-035045
TITLE: Preparing a DNA molecule comprising an amplifiable region, useful for producing a genomic **library**,

comprises subjecting the DNA molecules to primer extension/nick translation.

DERWENT CLASS: B04 D16
INVENTOR(S): LANGMORE, J P; MAKAROV, V
PATENT ASSIGNEE(S): (UNMI) UNIV MICHIGAN
COUNTRY COUNT: 95
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2001090415	A2	20011129	(200215)*	EN	372

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW

AU 2001074869 A 20011203 (200221)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2001090415	A2	WO 2001-US16264	20010518
AU 2001074869	A	AU 2001-74869	20010518

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2001074869 A	Based on	WO 200190415

PRIORITY APPLN. INFO: US 2000-206095P 20000520

AB WO 200190415 A UPAB: 20020306

NOVELTY - Preparing, (M1), a DNA molecule having an amplifiable region comprising subjecting the DNA molecules to nick translation comprising DNA polymerization and 5'-3' exonuclease activity to produce nick translate molecules, is new.

DETAILED DESCRIPTION - The method (M1) of preparing a DNA molecule having an amplifiable region comprises:

(a) obtaining a DNA sample comprising DNA molecules having regions to be amplified;

(b) attaching upstream adaptor molecules to ends of DNA molecules of the sample to provide a nick translation initiation site;

(c) subjecting the DNA molecules to nick translation comprising DNA polymerization and 5'-3' exonuclease activity to produce nick translate molecules; and

(d) attaching downstream adaptor molecules to the nick translate molecules to produce adaptor attached nick translate molecules.

INDEPENDENT CLAIMS are also included for the following:

(1) creating hybridization probes comprising preparing a labeled, amplified DNA;

(2) shotgun sequencing of DNA;

(3) constructing a genomic **library**;

(4) preparing an unordered DNA **library**;

(5) sequencing a BAC clone;

(6) kits comprising amplifiable DNA;

(7) an adaptor construct comprising:

(a) a first domain comprising nucleotides that facilitate ligation of the construct to a nucleic acid; and

(b) a second domain proximal to the first domain, comprising a site which facilitates the initiation of a nick translation reaction and a site that facilitates recombination, where ligation of the adaptor construct to

a **polynucleotide** results in the only free 3' OH group capable of initiating a nick translation reaction within the second domain;

(8) an adaptor construct comprising:

(a) a first oligonucleotide comprising a phosphate group at the **5' end** and a **blocking** nucleotide at the **3' end**;

(b) a second **oligonucleotide** comprising a **blocked 3' end**, a non-phosphorylated **5' end**, and a nucleotide sequence complementary to the 5' element of the first oligonucleotide; and

(c) a third oligonucleotide comprising a 3' hydroxyl group, a non-phosphorylated **5' end**, and a nucleotide sequence complementary to the 3' element of the first oligonucleotide;

(9) kits comprising a DNA polymerase, nucleotide triphosphates, and an adaptor construct;

(10) methods of recombining DNA molecules comprising recombining ends of adaptor attached template molecules in a dilute solution; and

(11) a method of detecting a specific DNA sequence.

USE - The method is useful in producing DNA to be sequenced or amplified with specific regions for which the sequence is not known, and in producing a genomic **library**. The method is useful for sequencing internal regions of short templates using primary and secondary PENTAmers, and complement PENTAmers; sequencing large insert clones using ordered positional **libraries** of PENTAmers; genomic sequencing; determining gene positions; sequencing and re-sequencing; cDNA sequencing; diagnosing chromosomal rearrangements; detecting and identifying organisms and variants of organisms; and amplifying specific subsets of genomes.

ADVANTAGE - In contrast to PCR, which amplified DNA between 2 specific sequences, Primer Extension/Nick Translation can amplify DNA between 2 specific positions. Positional amplification by nick translation is fast and economical, because PENTAmer preparation can be multiplexed.

Dwg.0/91

L169 ANSWER 24 OF 33 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 1994-255829 [32] WPIDS
DOC. NO. NON-CPI: N1994-201556
DOC. NO. CPI: C1994-116990
TITLE: **Polynucleotide** encoding a human excitatory amino acid 3 receptor or fragment - used to assay test ligands for their interaction with a human CBS receptor..
DERWENT CLASS: B04 D16 J04 S03
INVENTOR(S): ELLIOT, C E; KAMBOJ, R; NUTT, S L; ELLIOTT, C E
PATENT ASSIGNEE(S): (KAMB-I) KAMBOJ R; (ALLX) ALLELIX BIOPHARMACEUTICALS INC; (ELLI-I) ELLIOT C; (NUTT-I) NUTT S; (NUTT-I) NUTT S
COUNTRY COUNT: 20
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
CA 2110933	A	19940612	(199432)*		35
EP 617123	A1	19940928	(199437)	EN	22
R: AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE					
JP 06319550	A	19941122	(199506)		20
US 5547855	A	19960820	(199639)		39
EP 617123	B1	19990901	(199940)	EN	
R: AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE					
DE 69326228	E	19991007	(199947)		
US 6018023	A	20000125	(200012)		
ES 2138997	T3	20000201	(200013)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE

CA 2110933	A		CA 1993-2110933	19931208
EP 617123	A1		EP 1993-309956	19931210
JP 06319550	A		JP 1993-341263	19931210
US 5547855	A	Cont of	US 1992-989793	19921211
			US 1995-405392	19950315
EP 617123	B1		EP 1993-309956	19931210
DE 69326228	E		DE 1993-626228	19931210
			EP 1993-309956	19931210
US 6018023	A	Cont of	US 1992-989793	19921211
		Cont of	US 1995-405392	19950315
			US 1995-487691	19950607
ES 2138997	T3		EP 1993-309956	19931210

FILING DETAILS:

PATENT NO	KIND		PATENT NO
DE 69326228	E	Based on	EP 617123
US 6018023	A	Cont of	US 5547855
ES 2138997	T3	Based on	EP 617123

PRIORITY APPLN. INFO: US 1992-989793 19921211; US 1995-405392 19950315; US 1995-487691 19950607

AB CA 2110933 A UPAB: 19991122

Isolated **polynucleotide** (PN) encoding human excitatory amino acid (EAA) 3 receptor or a kainate-binding fragment is claimed.

Also claimed are: (1) a recombinant DNA construct contg. the PN; (2) a cell contg. the PN which produces a kainate-binding human EAA receptor, and a membrane prepn. derived from this cell; (3) a pure human EAA3 receptor or kainate binding fragment; (4) an antibody which binds a human EAA3 receptor; and (5) an **oligonucleotide** of at least 17 bases which hybridises with the PN.

The PN encodes human EAA3a, EAA3b or EAA3c receptor.

USE - The cell line expressing EAA receptor or its membrane prepn. can be used to assay test ligands for their interaction with a human CNS receptor by incubating the ligand with the cells and determining the **extent** of receptor binding. The antibody specific for the human EAA3 receptor can be covalently **coupled** to a receptor molecule, e.g. a radiolabel, and used as a specific probe for EAA3 receptors. Labelled PN can also be used as a hybridisation probe to identify sequence-related genes to mammalian genomes or cDNA **libraries**, or in specimens.

In an example, EAA3-encoding cDNA insert was released from pBS/humEAA3a as a 3.3 to NotI/NotI fragment subsequent to insertion of a HindIII/NotI adaptor at the 3' end of the insert. This fragment was then incorporated at the NOtI site in the PcDNAIA vector to form pcDNAIA/humEAA3a. This construct was used to transfer monkey-derived fibroblast-like cells of the COS-I lineage to express EAA3a receptor.

Dwg.0/6

L169 ANSWER 25 OF 33 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-17925 BIOTECHDS
TITLE: Amplifying nucleic acid by synthesizing template nucleic acid containing a predetermined sequence and hairpin structure and using the template for target amplification by Single Primer Amplification;
DNA amplification, nested oligonucleotide extension reaction and predetermined sequence
AUTHOR: BOWDISH K S; BARBAS-FREDERICKSON S; LIN Y; MCWHIRTER J; MARUYAMA T
PATENT ASSIGNEE: ALEXION PHARM INC
PATENT INFO: WO 2002048401 20 Jun 2002

APPLICATION INFO: WO 2000-US47727 11 Dec 2000
PRIORITY INFO: US 2001-323400 19 Sep 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-500537 [53]
AB DERWENT ABSTRACT:

NOVELTY - Amplifying (M1) nucleic acid using Single Primer Amplification (SPA) comprising synthesizing a template nucleic acid containing a predetermined sequence and hairpin structure with the nested oligonucleotide extension reaction, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) an engineered nucleic acid strand (I) comprising a predetermined sequence at a first end, a sequence complementary to the predetermined sequence at the other end, and a hairpin structure between them; and (2) a **library** (II) of polypeptides produced by (M1).

BIOTECHNOLOGY - Preferred Method: (M1) involves annealing a primer to a template nucleic acid sequence, where the primer has a first portion which anneals to the template and a second portion of predetermined sequence, synthesizing a **polynucleotide** that anneals to and is complementary to the portion of the template adjacent to the location at which the first portion of the primer anneals to the template, where the **polynucleotide** has a first end and a second end, where the first end incorporates the primer, separating the **polynucleotide** synthesized from the template, annealing a nested oligonucleotide having a first portion that anneals to the second end of the **polynucleotide**, and a second portion with a hairpin structure, extending the **polynucleotide** synthesized to provide an extended **polynucleotide** comprising a portion that is complementary to the hairpin structure and a terminal portion that is complementary to the predetermined sequence, and amplifying the extended **polynucleotide** using a single primer with the predetermined sequence. (M1) also involves providing a nucleic acid template by annealing a restriction oligonucleotide to a nucleic acid strand to form a double stranded portion and digesting the nucleic acid strand at the double stranded position. The template is selected from full length or truncated messenger ribonucleic acid (mRNA), deoxyribonucleic acid (DNA) or complementary DNA (cDNA), and encodes an immunoglobulin or its fragment. The nucleic acid being amplified includes a target sequence encoding a polypeptide, where the target sequence encodes an immunoglobulin or its fragment. (M1) further comprises digesting the extended **polynucleotide** to isolate the target sequence, ligating the isolated target sequence into an expression vector, transforming a host cell with the expression vector and expressing the polypeptide encoded by the target sequence. (M1) involves annealing a primer, preferably an oligo dT primer and a boundary oligonucleotide to a template nucleic acid sequence, preferably an mRNA template. (M1) further comprises providing a nucleic acid template by generating first strand cDNA from mRNA. In (M1), the steps of annealing, synthesizing **polynucleotides** and separating nucleotides are repeated 15-25 times prior to annealing the nested oligonucleotide. Preferred **Library**: (II) comprises at least a portion of antibodies.

USE - (M1) is useful for amplifying a nucleic acid, preferably for amplifying a family of related nucleic acid sequences to build a complex **library** of polypeptides encoded by the sequences. (I) is useful for amplifying a nucleic acid strand by providing a nucleic acid strand with a predetermined sequence engineered onto its first end, a sequence complementary to the predetermined sequence and a hairpin structure between them and contacting the engineered nucleic acid strand with a primer containing at least a portion of the predetermined sequence in the presence of a polymerase and nucleotides under conditions suitable for polymerization to produce a complementary nucleic acid strand (claimed). (M1) is useful for producing large amounts of a target nucleic acid sequence and for amplifying simultaneously more than one different target

nucleic acid sequence located on the same or different nucleic acid molecules.

EXAMPLE - Amplification of a repertoire of 1 g kappa light chain variable genes was as follows. First strand complementary deoxyribonucleic acid (cDNA) to be used as the original template was generated from 2 mug of human peripheral blood lymphocyte (PBL) messenger ribonucleic acid (mRNA) with an oligo-dT primer. The first strand cDNA product was purified and eluted. Second strand linear amplification (SSLA) was performed in the presence of **blocking oligonucleotide**. The second strand cDNA reaction contained 5 mul of first strand cDNA original template, 0.5 muM primer JMX26VK1a (i), where R is an equal mixture of A and G, 0.5 muM blocking oligo CKLNA1 (ii), 0.2 mM dNTPs, 5 units of AmpliTaq Gold DNA polymerase, 1x GeneAmp Gold Buffer and 1.5 mM MgCl2. After an initial heat denaturation linear amplification of second strand cDNA was carried out. After the last cycle of linear amplification, 2 mul of a nested/hairpin oligo designated JK14TSHP (iii) was added to give a final concentration of 20 muM. Y is an equal mixture of C and T and (ps) were phosphorothioate backbone linkages and P is a 3' propyl group. The reaction products were purified and eluted. The efficiency of the nested oligo extension reaction was determined by amplifying the products with either a primer set specific for the engineered product or a primer set that detected all VK1a/JK14 second strand cDNA products. For specific detection of engineered product, a 10 mul aliquot was amplified with primers designated JMX26 (iv) and TSDP (v). For detection of all VK1a/JK14 second strand cDNA products a 10 mul aliquot was amplified with primers JMX26 and JK14 (vi). The results with primers JMX26 and TSDP demonstrated the successful production of nested oligo and extended VK stem-loop DNA when using SSLA DNA that was blocked specifically with a boundary oligo. Single primer amplification of the stem-loop cDNA template was performed. Amplified fragments were cloned by SacI/AscI into an appropriate expression vector that contained, in frame, the remaining portion of the kappa constant region. Suitable vectors include pRL5 and pRL4 vectors fdtetDOG, PHEN1, and pCANTAB5E. Individual kappa clones were sequenced and the repertoire of VKappa amplified products were expanded. 5'-GTCACTCACGAACTCACGACTCACGGAGAGCTCRACATCCAGATGACCCAG-3' (i); 5'-GAACTGTGGCTGCACCATCTG-3' (ii); 5'-CCTTAGAGTCACGCTAGCGATTGATTGATTGATTGATTGTTTGTTGTGACTCTAAGGTTGGCGCGCCTTCGTTTGATYTCCACCTTGGTCC(ps)T(ps)P-3' (iii); 5'-GTCACTCACGAACTCACGATCACGG-3' (iv); 5'-CACGCTAGCGATTGATTGATTG-3' (v); and 5'-GAGGAGGAGGAGGAGGAGGGCGCGCCTGATYTCCACCTTGGTCCC-3' (vi).(54 pages)

L169 ANSWER 26 OF 33 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-19584 BIOTECHDS
TITLE: Novel isolated human G-protein **coupled** receptor protein useful for diagnosing, treating, preventing hypertension, myocardial infarction, anorexia, cholecystitis, anemia, asthma, diabetes, obesity, Alzheimer's disease; vector-mediated recombinant protein gene transfer and expression in host cell for use in diagnosis and gene therapy
AUTHOR: KALLICK D A; BAUGHN M R; LU D A M; YUE H; GRAUL R C; LU Y; DING L; TRIBOULEY C M; TANG Y T; GANDHI A R; THORNTON M
PATENT ASSIGNEE: INCYTE GENOMICS INC
PATENT INFO: WO 2002046230 13 Jun 2002
APPLICATION INFO: WO 2000-US46659 8 Dec 2000
PRIORITY INFO: US 2001-262848 19 Jan 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-519657 [55]
AB DERWENT ABSTRACT:
NOVELTY - Isolated human G-protein **coupled** receptor involved in olfactory and/or taste sensation (referred as GCREC 1-11) (I) having a 1018, 309, 319, 309, 314, 317, 312, 311, 316, 316 or 314 residue amino

acid sequence (PS), given in specification, or naturally occurring polypeptide having 90 % sequence identity to PS, or biologically active or immunogenic fragment of PS, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an isolated polynucleotide (II) encoding (I), where (II) comprises a 3486, 1010, 960, 1801, 1205, 1050, 939, 939, 951, 971, or 1092 nucleotide sequence (NS), given in the specification, is a naturally occurring polynucleotide sequence having 90 % identity to NS, a polynucleotide sequence which is complementary to the polynucleotide sequences, or is a RNA equivalent of the sequences; (2) a recombinant polynucleotide (III) comprising a promoter sequence operably linked to (II); (3) a cell (IV) transformed with (III); (4) a transgenic organism comprising (III); (5) preparing (I), comprising culturing (IV) under expression conditions, and recovering the polypeptide; (6) an isolated antibody (V) that specifically binds to (I); (7) detecting (D) a (II) in sample, comprising: (a) hybridizing the sample with a probe comprising at least 20 contiguous nucleotides which is complementary to, and specifically hybridizes to the target polynucleotide, to form a hybridization complex; (b) detecting the complex formed; and (c) optionally quantifying the amount of target polynucleotide; (8) detecting (D) a (II) in sample, comprising: (a) amplifying the target polynucleotide or its fragments; (b) detecting the presence or absence of the amplified target polynucleotide or its fragment; and (c) optionally, quantifying the amount of the target polynucleotide; (9) an isolated polynucleotide comprising 60 contiguous nucleotides of (II); (10) a composition (C1) comprising (I) and an excipient; (11) a composition (C2) comprising the agonist or antagonist identified using (I); (12) a composition (VI) comprising (V); (13) a monoclonal or polyclonal antibody (VII) having specificity of (V), produced using (I); (14) a composition comprising (VII) and a carrier; (15) a microarray (VIII) comprising (II) as an element of the microarray; and (16) an array (IX) comprising different nucleotide molecules affixed in distinct physical locations on a solid substrate, where at least one of the nucleotide molecules comprises a first **oligonucleotide** or polynucleotide sequence specifically hybridizable with at least 30 contiguous nucleotide of a target polynucleotide which is (II).

WIDER DISCLOSURE - (1) polynucleotide sequences that are capable of hybridizing to (II); and (2) sequences which differ from (II) due to degeneracy of genetic code.

BIOTECHNOLOGY - Preferred Molecules: (I) has a polypeptide sequence of any one of PS, and (II) preferably has a polynucleotide sequence of any one of NS. Preferred Antibody: (V) is produced by screening a Fab expression **library** or by screening a recombinant immunoglobulin **library**. Preferred Method: In (D), the probe which is complementary to the target polynucleotide having a sequence of (II), preferably comprises at least 60 contiguous nucleotides. Preferred Array: (IX) comprises at least one nucleotide molecule comprising a first **oligonucleotide** or polynucleotide sequence which is completely complementary to at least 30, preferably 60 contiguous nucleotides of target polynucleotide, or is completely complementary to the target nucleotide. The array is preferably a microarray, and further comprises target polynucleotide hybridized to nucleotide molecule comprising the first **oligonucleotide** or polynucleotide sequence. In the array, a linker joins at least one of the nucleotide molecules to the solid substrate, and each distinct physical location the substrate contains multiple nucleotide molecules, and the multiple nucleotide molecules at any single distinct physical location have the same sequence, and each distinct physical location on the substrate contains nucleotide molecules having a sequence differing from the sequence of nucleotide molecules at another distinct physical location on the substrate.

ACTIVITY - Cytostatic; Anticonvulsant; Nootropic; Neuroprotective; Hypotensive; Antianginal; Cardiant; Antiinflammatory; Hepatotropic; Anti-HIV (human immunodeficiency virus); Antianemic; Antiasthmatic;

Antidiabetic; Anorectic; Virucide. No biological data is given.

MECHANISM OF ACTION - GCREC 1-11 expression modulators; Gene therapy; GCREC 1-11 agonist or antagonist.

USE - (I) is useful for screening a compound for effectiveness as agonist or antagonist of (I), that specifically binds to (I), and that modulates the activity of (I). (I) is also useful for preparing a polyclonal or monoclonal antibody with specificity of (V). (I) is useful for identifying a compound that modulates, mimics and/or blocks an olfactory and/or taste sensation. (II) is useful for screening a compound for effectiveness in altering expression of a target polynucleotide (i.e. a polynucleotide having a sequence of NS). (II) is useful for assessing toxicity of a test compound which involves treating a biological sample containing nucleic acids with the test compound, hybridizing the nucleic acids of the treated biological sample with a probe comprising at least 20 contiguous nucleotides of (II) to form a specific hybridization complex between probe and target polynucleotide which comprises a sequence of (II) or its fragment, quantifying amount of hybridization complex, and comparing amount of hybridization complex in treated biological sample with amount of hybridization complex in an untreated biological sample, where a difference in the amount of hybridization complex in treated biological sample is indicative of toxicity of the test compound. (IV) is useful for producing (I) by recombinant techniques. (V) (a chimeric or single-chain antibody, Fab fragment, a F(ab')2 fragment, or a humanized antibody) is useful in a diagnostic test for a condition or disease associated with the expression of GCREC 1-11 in a biological sample. (V) is also useful for detecting (I) in a sample or purifying (I) from a sample. (VI) is useful for diagnosing a condition or disease associated with the expression of GCREC 1-11 in a subject. Preferably, the antibody in the composition is labeled. (C1), and (C2) which comprises an agonist of (I) are useful for treating a disease or condition associated with decreased expression of functional GCREC 1-11. (C2) comprising antagonist of (I) is useful for treating a disease or condition associated with overexpression of (I). (VIII) is useful for generating expression profile of a sample which contains polynucleotides. (All claimed). (I) and (II) are useful in the diagnosis, treatment and prevention of a cell proliferative disorder (e.g. actinic keratosis, leukemia, etc.), a neurological disorder (e.g. epilepsy, Alzheimer's disease, etc.), a cardiovascular disorder (e.g. hypertension, angina pectoris, myocardial infarction, etc.), a gastrointestinal disorder (e.g. anorexia, cholecystitis, Crohn's disease, etc.), an autoimmune/inflammatory disorder (e.g. acquired immunodeficiency syndrome, anemia, asthma, etc.), a metabolic disorder (e.g. diabetes, obesity, etc.), and an infection by a viral agent such as adenovirus, arenavirus, etc. (I) may be used in assays for detecting the presence of the associated disorders. (II) is useful for detecting upstream sequences such as promoters and regulatory elements, creating knock out or knock in humanized animals or transgenic animals to model human diseases, and somatic or germline gene therapy for treating the above mentioned disorders. (II) may also be used for generating hybridization probes useful in mapping the naturally occurring genomic sequences, and for developing genetic linkage maps, detecting differences in chromosomal location due to translocation, inversion etc. (II) is also useful for generating a transcript image of a tissue or cell type.

ADMINISTRATION - The pharmaceutical compositions are administered by oral, intravenous, intramuscular, intraarterial, intramedullary, intrathecal, intraventricular, pulmonary, transdermal, or subcutaneous routes, etc. The dosage is 0.1-100000 micro-g.

EXAMPLE - Incyte cDNAs were derived from cDNA **libraries** described in LIFESEQ GOLD database and as given in the specification, or cDNA **libraries** were constructed from cDNA obtained from RNA isolated by homogenizing certain tissues. Plasmids comprising cDNAs were recovered from Escherichia coli cells by in vivo excision, and purified. Incyte cDNAs recovered in plasmids were sequenced. The polynucleotide

sequences derived from incyte cDNAs were validated by removing vector, linker and poly(A) sequences using algorithms and programs based on BLAST, dynamic programming, and dinucleotide nearest neighbor analysis. The Incyte cDNA sequences or translations of it were queried against a selection of public databases. The Incyte cDNA sequences were **assembled** to produce full length **polynucleotide** sequences. The full length polynucleotide sequences were translated to derive the corresponding full length polypeptide sequences. Full length polypeptide sequences were subsequently analyzed by querying against databases such as the GenBank protein database, SwissProt, BLOCKS, PRINTS, DOMO, PRODOM, Prosite, and hidden Markov model (HMM)-based protein family databases such as PFAM. Putative G-protein **coupled** receptors were initially identified by running the Genscan gene identification program against public genomic sequence databases. Full length **polynucleotide** sequences were obtained by **assembling** Genscan-predicted coding sequences with Incyte cDNA sequences and/or public cDNA sequences using the assembly process. Assembly of genomic sequence data with cDNA sequence data comprises stitched sequences and stretched sequences. Partial cDNAs assembled as described above were mapped to genomic DNA and parsed into clusters containing related cDNAs and Genscan exon predictions from one or more genomic sequences. Each cluster was analyzed using an algorithm based on graph theory and dynamic programming to integrate cDNA and genomic information, generating possible splice variants that were subsequently confirmed, edited, or **extended** to create a full length sequence. Sequence intervals in which the entire length of the interval was present on more than one sequence in the cluster were identified. Intervals thus identified were stitched together by the stitching algorithm in the order that they appeared along their parent sequences to generate the longest possible sequence. Linkages between intervals which proceed along one type of parent sequence (cDNA to cDNA or genomic sequence to genomic sequence) were given preference over linkages which change parent type (cDNA to genomic sequence). The resultant stitched sequences were translated and compared by BLAST analysis to the genpept and gbpri public databases. Partial cDNAs assembled were queried against public databases using the BLAST program. The nearest GenBank protein homolog was compared by the BLAST analysis to either Incyte cDNA sequences or GenScan exon predicted sequences described above. A chimeric protein was generated by using the resultant high-scoring segment pairs (HSPs) to map the translated sequences onto the GenBank protein homolog. The GenBank protein homolog, the chimeric protein, or both were used as probes to search for homologous genomic sequences from the public human genome databases. Partial DNA sequences were therefore stretched or **extended** by the addition of homologous genomic databases. The resultant stretched sequences were examined to determine whether it contained a complete gene.(136 pages)

L169 ANSWER 27 OF 33 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-12761 BIOTECHDS
TITLE: G-protein **coupled** receptor polynucleotides and polypeptides are useful in the diagnosis, treatment and prevention of cell proliferative, neurological, cardiovascular, gastrointestinal and viral infections; vector-mediated recombinant protein gene transfer and expression in host cell, transgenic animal, hybridoma cell culture for monoclonal antibody and chimeric antibody, single chain antibody, Fab fragment, F(ab')2 fragment, humanized antibody production, DNA microarray for autoimmune, inflammatory, metabolic disorder therapy
AUTHOR: KALLICK D A; LEE E A; TRIBOULEY C M
PATENT ASSIGNEE: INCYTE GENOMICS INC
PATENT INFO: WO 2002029061 11 Apr 2002
APPLICATION INFO: WO 2000-US42541 6 Oct 2000

PRIORITY INFO: US 2000-238394 6 Oct 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-315863 [35]
AB DERWENT ABSTRACT:
NOVELTY - A human G-protein **coupled** receptor (GPCR), is new.
DETAILED DESCRIPTION - A human G-protein **coupled** receptor (GPCR), is new. The GPCR polypeptide (P1) is selected from: (a) a polypeptide comprising the 309 amino acid sequence (S1) defined in the specification; (b) a polypeptide comprising a naturally occurring amino acid sequence having at least 90% sequence identity to S1; (c) a biologically active fragment of S1; or (d) an immunogenic fragment of S1. INDEPENDENT CLAIMS are included for the following: (1) an isolated polynucleotide (N1) encoding P1; (2) a recombinant polynucleotide (N2) comprising a promoter sequence operably linked to N1; (3) a cell transformed N2; (4) a transgenic organism comprising N2; (5) a method for producing P1, comprising culturing a cell under conditions suitable for expression of the polypeptide, where the cell is transformed with a recombinant polynucleotide, and the recombinant polynucleotide comprises a promoter sequence operably linked to a polynucleotide encoding the P1, and recovering the polypeptide so expressed; (6) an isolated antibody (Ab1) which specifically binds to a P1; (7) an isolated polynucleotide (N3) selected from: (a) a polynucleotide comprising the 948 nucleotide sequence (S2) defined in the specification; (b) a polynucleotide comprising a naturally occurring polynucleotide sequence having at least 90% sequence identity to S2; (c) a polynucleotide sequence complementary to the polynucleotide (a) or (b); or (d) an RNA equivalent of the polynucleotide of (a)-(c); (8) an isolated polynucleotide comprising at least 60 contiguous nucleotides of N3; (9) a method (M1) for detecting a target polynucleotide in a sample, the target polynucleotide having a sequence of N3; (10) a method for treating a disease or condition associated with decreased expression of functional GPCR, comprising administering to a patient in need of such treatment a composition comprising P1; (11) a method (M2) for screening a compound for effectiveness as an agonist of P1, comprising exposing a sample comprising P1 to a compound, and detecting agonist activity in the sample; (12) a composition (C1) comprising an agonist compound identified by M2; (13) a method for treating a disease or condition associated with decreased expression of functional GPCR, comprising administering to a patient in need of such treatment the composition of C1; (14) a method (M3) for screening a compound for effectiveness as an antagonist of P1, comprising exposing a sample comprising P1 to a compound, and detecting antagonist activity in the sample; (15) a composition (C2) comprising an antagonist compound identified by M3; (16) a method for treating a disease or condition associated with overexpression of functional GPCR, comprising administering to a patient in need of such treatment the composition of C2; (17) a method of screening for a compound that specifically binds to P1, comprising combining P1 with at least one test compound under suitable conditions, and detecting binding of P1 to the test compound, therefore identifying a compound that specifically binds to the P1; (18) a method (M4) of screening for a compound that modulates the activity of P1; (19) a method (M5) for screening a compound for effectiveness in altering expression of a target polynucleotide, where the target polynucleotide comprises N1; (20) a method (M6) for assessing toxicity of a test compound; (21) a diagnostic test (M7) for a condition or disease associated with the expression of GPCR in a biological sample; (22) a method of diagnosing a condition or disease associated with the expression of GPCR in a subject, comprising administering to the subject an effective amount of labeled Ab1; (23) a method (M8) of preparing a polyclonal antibody with the specificity of Ab1, comprising immunizing an animal with a polypeptide having the sequence of S1, or its immunogenic fragment, under conditions suitable for eliciting an antibody response, isolating antibodies from the animal, and screening the isolated

antibodies with the polypeptide, therefore identifying a polyclonal antibody which specifically binds to the polypeptide; (24) a method (M9) of preparing a monoclonal antibody with the specificity of Ab1; (25) an antibody produced by M8 or M9; (26) a method (M10) for detecting a polypeptide having the sequence of S1 in a sample; (27) a method (M11) of purifying a polypeptide comprising the amino acid sequence of S1 from a sample; (28) a microarray where at least one element of the microarray is N3;(29) a method (M12) of generating a transcript image of a sample which contains polynucleotides; (30) an array (A1) comprising different nucleotide molecules affixed in distinct physical locations on a solid substrate; and (31) a method (M13) of identifying a compound that modulates, mimics and/or blocks an olfactory and/or taste sensation.

BIOTECHNOLOGY - Preferred Polypeptide: Preferably, P1 comprises the sequence of S1. Preferred Polynucleotide: N1 comprises the sequence of S2. Preferred Method: M1 comprises: (a) hybridizing the sample with a probe comprising at least 20 contiguous nucleotides comprising a sequence complementary to the target polynucleotide in the sample, and which probe specifically hybridizes to the target polynucleotide, under conditions where a hybridization complex is formed between the probe and the target polynucleotide or its fragments; and (b) detecting the presence or absence of the hybridization complex, and, optionally, if present, its amounts. In M1, the probe comprises at least 60 contiguous nucleotides. Alternatively, M1 comprises amplifying the target polynucleotide or its fragment using polymerase chain reaction amplification, and detecting the presence or absence of the amplified target polynucleotide or its fragment, and, optionally, if present, its amount. M4 comprises combining P1 with at least one test compound under conditions permissive for the activity of the P1, assessing the activity of P1 in the presence of the test compound, and comparing the activity of P1 in the presence of the test compound with the activity of the P1 in the absence of the test compound, where a change in the activity of the P1 in the presence of the test compound is indicative of a compound that modulates the activity of P1. M5 comprises exposing a sample comprising the target polynucleotide to a compound, under conditions suitable for the expression of the target polynucleotide, detecting altered expression of the target polynucleotide, and comparing the expression of the target polynucleotide in the presence of varying amounts of the compound and in the absence of the compound. M6 comprises: (a) treating a biological sample containing nucleic acids with the test compound; (b) hybridizing the nucleic acids of the treated biological sample with a probe comprising at least 20 contiguous nucleotides of N3 under conditions where a specific hybridization complex is formed between the probe and a target polynucleotide in the biological sample, the target polynucleotide comprising a polynucleotide sequence of N3 or its fragment; (c) quantifying the amount of hybridization complex; and (d) comparing the amount of hybridization complex in the treated biological sample with the amount of hybridization complex in an untreated biological sample, where a difference in the amount of hybridization complex in the treated biological sample is indicative of toxicity of the test compound. M7 comprises combining the biological sample with Ab1, under conditions suitable for the antibody to bind the polypeptide and form an antibody:polypeptide complex; and detecting the complex, where the presence of the complex correlates with the presence of the polypeptide in the biological sample. M9 comprises immunizing an animal with a polypeptide having the sequence of S1, or its immunogenic fragment, under conditions suitable for eliciting an antibody response, isolating antibody-producing cells from the animal, fusing the antibody-producing cells with immortalized cells in culture to form monoclonal antibody-producing hybridoma cells, culturing the hybridoma cells, and isolating from the culture monoclonal antibodies which specifically bind to the polypeptide. M10 comprises combining the sample with an antibody which specifically binds to the polypeptide under conditions suitable for specific binding between the antibody and the polypeptide, and detecting

specific binding, where specific binding indicates the presence of the polypeptide in the sample. M11 comprises incubating Ab1 with a sample under conditions to allow specific binding of the antibody and the polypeptide, and separating the antibody from the sample and obtaining the purified polypeptide. M12 comprises labeling the polynucleotides of the sample, contacting the elements of the microarray of (28) with the labeled polynucleotides of the sample under conditions suitable for the formation of a hybridization complex, and quantifying the expression of the polynucleotides in the sample. M13 comprises: (a) contacting the compound with an olfactory arid/or taste receptor polypeptide selected from: (i) a polypeptide having the amino acid sequence of S1; (ii) a biologically active fragment of a polypeptide having the amino acid sequence of S1; and (iii) an olfactory and/or taste receptor having an amino acid sequence at least 90% identical to the amino acid sequence of S1; (b) identifying whether the compound specifically binds to and/or affects the activity of the receptor polypeptide. The receptor polypeptide is expressed on the surface of a mammalian cell which expresses a G-protein(s). The mammalian cell expresses another olfactory and/or taste receptor polypeptide. The receptor polypeptide is fused to another polypeptide. Preferred Antibody: Ab1 is a chimeric antibody, a single chain antibody, a Fab fragment, a F(ab')2 fragment, or a humanized antibody. Ab1 is produced by screening a Fab expression **library** or a recombinant immunoglobulin **library**. Preferred Array: In A1, at least one of the nucleotide molecules comprises a first **oligonucleotide** or polynucleotide sequence specifically hybridizable with at least 30 contiguous nucleotides of a target polynucleotide, and where the target polynucleotide is N3. The first **oligonucleotide** or polynucleotide sequence is completely complementary to at least 30 or 60 contiguous nucleotides of the target polynucleotide. Alternatively, the first **oligonucleotide** or polynucleotide sequence is completely complementary to the target polynucleotide. A1 is a microarray. A1 further comprises the target polynucleotide hybridized to a nucleotide molecule comprising the first **oligonucleotide** or polynucleotide sequence. The linker joins at least one of the nucleotide molecules to the solid substrate. Each distinct physical location on the substrate contains multiple nucleotide molecules, and the multiple nucleotide molecules at any single distinct physical location have the same sequence, and each distinct physical location on the substrate contains nucleotide molecules having a sequence which differs from the sequence of nucleotide molecules at another distinct physical location on the substrate. Preparation: The GPCR polypeptides are produces using standard genetic engineering techniques.

ACTIVITY - Antiinflammatory; cytostatic; nootropic; neuroprotective; antiallergic; antiviral; antidiabetic; cardiant; antiparkinsonian; anoretic; appetite stimulant; antiarthritic; antiviral; anticonvulsant; immunomodulatory. No biological data given.

MECHANISM OF ACTION - GPCR agonist; GPCR antagonist. No biological data given.

USE - The GPCR polynucleotides and polypeptides are useful in the diagnosis, treatment and prevention of cell proliferative (e.g. actinic keratosis, arteriosclerosis, atherosclerosis, bursitis, cirrhosis, hepatitis, and cancers including adenocarcinoma, leukemia, lymphoma, and melanoma), neurological (e.g. epilepsy, Alzheimer's disease, Pick's disease, Huntington's disease, dementia, Parkinson's disease), cardiovascular (e.g. such as arteriovenous fistula, atherosclerosis, hypertension, vasculitis, Rnynaud's disease, aneurysms, arterial, vascular tumors, ischemic heart disease, angina pectoris, myocardial infarction, and hypertensive heart disease), gastrointestinal (e.g. dysphagia, peptic esophagitis, esophageal spasm, esophageal carcinoma, dyspepsia, indigestion, gastritis, gastric carcinoma, anorexia), autoimmune/inflammatory (e.g. acquired immunodeficiency syndrome (AIDS), Addison's disease, adult respiratory distress syndrome and allergies), metabolic disorders (e.g. diabetes, obesity, and osteoporosis) and viral

infections. Numerous other examples of each disorder are given in the specification.

ADMINISTRATION - The GPCR polynucleotides, GPCR polypeptides, GPCR agonists and GPCR antagonists are administered by oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, pulmonary, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual or rectal means. The dosage amounts vary from 0.1 micrograms to 1 gm, depending on the route of administration.

EXAMPLE - Incyte cDNAs were derived from cDNA **libraries** described in the LIFESEQ GOLD database (Incyte Genomics, Palo Alto CA) using standard genetic techniques. Incyte cDNA recovered in plasmids were sequenced as follows. Sequencing reactions were processed using standard methods or high-throughput instrumentation such as the ABI CATALYST 800 (Applied Biosystems) thermal cycler or the PTC-200 thermal cycler (MI Research) in conjunction with the HYDRA microdispenser (Robbins Scientific) or the MICROLAB 2200 (Hamilton) liquid transfer system. cDNA sequencing reactions were prepared using reagents provided by Amersham Pharrnacia Biotech or supplied in ABI sequencing kits such as the ABI PRISM BIGDYE Terminator cycle sequencing ready reaction kit (Applied Biosystems). Electrophoretic separation of cDNA sequencing reactions and detection of labeled polynucleotides were carried out using the MEGABACE 1000 DNA sequencing system (Molecular Dynamics); the ABI PRISM 373 or 377 sequencing system (Applied Biosystems) in conjunction with standard ABI protocols and base calling software; or other sequence analysis systems known in the art. Reading frames within the cDNA sequences were identified using standard methods. Some of the cDNA sequences were selected for **extension**. The polynucleotide sequences derived from Incyte cDNAs were validated by removing vector, linker, and poly(A) sequences and by masking ambiguous bases, using algorithms and programs based on BLAST, dynamic programming, and dinucleotide nearest neighbor analysis. The Incyte cDNA sequences or its translations were then queried against a selection of public databases such as the GenBank primate, rodent, mammalian, vertebrate, and eukaryote databases, and BLOCKS, PRINTS, DOMO, PRODOM, and hidden Markov model (HMM)-based protein family databases such as PFAM. (HMM is a probabilistic approach which analyzes consensus primary structures of gene families. See, for example, Eddy, S.R. (1996) Curr. Opin. Struct. Biol. 6:361-365). The queries were performed using programs based on BLAST, FASTA, BLIMPS, and HMMER. The Incyte cDNA sequences were **assembled** to produce full length **polynucleotide** sequences. Alternatively, GenBank cDNAs, GenBank ESTs, stitched sequences, stretched sequences, or 15 Genscan-predicted coding sequences were used to **extend** Incyte cDNA. The methods were used to identify polynucleotide sequence fragments from the 948 nucleotide sequence (S2) defined in the specification.(98 pages)

L169 ANSWER 28 OF 33 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-15402 BIOTECHDS
TITLE: Novel G-protein **coupled** receptor polypeptides referred as GCREC peptides, useful for treating multiple sclerosis, cholecystitis heart failure, angina pectoris, rheumatoid arthritis, obesity, osteoporosis; vector-mediated recombinant protein gene transfer and expression in host cell, monoclonal antibody and DNA microarray for use in drug screening and cancer, neurological disease, cardiovascular disease, gastrointestinal disease, autoimmune disease, inflammatory disease and metabolic disorder gene therapy
AUTHOR: BAUGHN M R; GRAUL R C; WALIA N K; GANDHI A R; HAFALIA A J A; RAMKUMAR J; TRIBOULEY C M; THORNTON M; KALLICK D A; YAO M G; ELLIOTT V S; BURFORD N; KHAN F A; YUE H; LU Y; ARVIZU C; ROOPA R; NGUYEN D B; LEE E A; LU D A M; ISON C H; WALSH R T; POLICKY J L

PATENT ASSIGNEE: INCYTE GENOMICS INC
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LANGUAGE: English
OTHER SOURCE: WPI: 2002-426012 [45]

AB DERWENT ABSTRACT:

NOVELTY - Isolated human G-protein **coupled** receptor (referred as GCREC 1-16) polypeptide (I) having a fully defined sequence (PS) of 217, 578, 441, 797, 434, 339, 549, 188, 332, 948, 315, 312, 309, 309, 315 or 307 amino acids (S1-S16) as given in specification, or a naturally occurring polypeptide comprising an amino acid sequence having 90% sequence identity to PS, or biologically active or immunogenic fragments of PS, are new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an isolated polynucleotide (II) encoding (I). (II) comprises a fully defined sequence (NS) of 2422, 1912, 1326, 3058, 1993, 1499, 2455, 2056, 999, 3429, 948, 939, 930, 1161, 948 or 924 nucleotides as given in specification, is a naturally occurring polynucleotide sequence having 90% identity to NS, a polynucleotide sequence which is complementary to the above mentioned polynucleotide sequences, or is a RNA equivalent of the above mentioned sequences; (2) a recombinant polynucleotide (III) comprising a promoter sequence operably linked to (II); (3) a cell (IV) transformed with (III); (4) a transgenic organism comprising (III); (5) preparation of (I); (6) an isolated antibody (V) that specifically binds to (I); (7) detecting (D) a target polynucleotide in a sample which comprises a sequence of (II) involves hybridizing the sample with a probe comprising at least 20 contiguous nucleotides which is complementary to, and specifically hybridizes to the target polynucleotide, to form a hybridization complex which is then detected. Optionally, the amount of the target polynucleotide is also quantitated. Alternately, the method is carried out by amplifying the target polynucleotide or its fragments and then detecting the presence or absence of the amplified target polynucleotide or its fragment, and, optionally, if present the amount of the target polynucleotide is also quantitated; (8) an isolated polynucleotide (N1) comprising 60 contiguous nucleotides of (II); (9) a composition (C1) comprising (I) and an excipient; (10) a composition (C2) comprising the agonist or antagonist identified using (I); (11) a composition (VI) comprising (V); (12) a monoclonal or polyclonal antibody (VII) produced (M1 and M2) using (I), having a specificity of (V); (13) a composition comprising (VII) and a carrier; (14) a microarray (VIII), where at least one element of the microarray is N1; and (15) an array (IX) comprising different nucleotide molecules affixed in distinct physical locations on a soluble substrate, where at least one of the nucleotide molecules comprise a first **oligonucleotide** or polynucleotide sequence specifically hybridizable with 30 contiguous nucleotides of a target polynucleotide, which is (II).

WIDER DISCLOSURE - The following are also disclosed: (1) polynucleotide sequences that are capable of hybridizing to (II); and (2) sequences which differ from (II) due to degeneracy of genetic code.

BIOTECHNOLOGY - Preparation: Preparation of (I) is by standard recombinant techniques (claimed). Preferred Molecules: (I) preferably has any one of the polypeptide sequence of PS, and (II) preferably has any one of the polynucleotide sequence of NS. Preferred Antibody: (V) is produced by screening a Fab expression **library** or by screening a recombinant immunoglobulin **library**. Preferred Methods: In (D), the probe which is complementary to the target polynucleotide having a sequence of (II), preferably comprises at least 60 contiguous nucleotides. In (M1) a monoclonal antibody is made by: (1) immunizing an animal with (I), or an immunogenic fragment, to elicit an immune response; (2) isolating antibody producing cells from the animal; (3)

fusing the cells with immortalized cells to form monoclonal antibody-producing hybridoma cells; (4) culturing the hybridoma cells; and (5) isolating the monoclonal antibody which binds specifically to (I), from the culture. (M2), production of a polyclonal antibody, comprises steps (2, 3 and 5) above. Preferred Array: (IX) comprises different nucleotide molecules affixed at specific positions on a solid substrate, where one of the nucleotide molecules comprise a first **oligonucleotide** or polynucleotide sequence which is completely complementary to 30 (preferably 60) contiguous nucleotides of (II). Optionally, the first **oligonucleotide** or polynucleotide sequence is completely complementary to (II). (IX) is preferably a microarray, and the array further comprises the target polynucleotide hybridized to a nucleotide molecule comprising the first **oligonucleotide** or polynucleotide sequence. A linker joins at least one of the nucleotide molecules to the solid substrate. Each distinct physical location on the substrate contains multiple nucleotide molecules which have the same sequence at any single distinct physical location, where each distinct physical location contains nucleotide molecules having a sequence which differs from the sequence of nucleotide molecules at another distinct physical location on the substrate.

ACTIVITY - Antiatherosclerotic; hepatotrophic; neuroprotective; anticonvulsant; nootropic; antianginal; cardiant; metabolic; antiinflammatory; antirheumatic; antiarthritic; immunosuppressive; thyromimetic; anorectic; osteopathic. No supporting data is given.

MECHANISM OF ACTION - GCREC 1-16 expression modulators; gene therapy; GCREC 1-16 agonist or antagonist.

USE - (I) is useful for screening a compound (i) for effectiveness as agonist or antagonist of (I); (ii) that specifically binds to (I); and (iii) that modulates the activity of (I). (I) is useful for preparing a polyclonal or monoclonal antibody with specificity of (V). (I) is useful for identifying a compound that modulates, mimics and/or blocks olfactory and/or taste sensation which involves contacting the compound with olfactory and/or taste receptor polypeptide such as (I) having a sequence of (S1)-(S16), or its biologically active fragment; or an olfactory and/or taste receptor having an amino acid sequence at least 90% identical to (S1)-(S15) or (S16); and identifying whether the compound specifically binds to and/or affects the activity of the receptor polypeptide which is preferably expressed on the surface of a mammalian cell. The mammalian cell also expresses a G-protein and several G-protein **coupled** receptors and another olfactory and/or taste receptor polypeptide. The receptor polypeptide employed in the method is preferably fused to another polypeptide. (II) is useful for screening a compound for effectiveness in altering expression of a target polynucleotide which comprises a sequence of NS. (II) is also useful for assessing toxicity of a test compound which involves treating a biological sample containing nucleic acids with the test compound, hybridizing the nucleic acids of the treated biological sample with a probe comprising at least 20 contiguous nucleotides of (II) to form a specific hybridization complex between probe and target polynucleotide comprising a polynucleotide sequence of (II) or its fragment, in the biological sample, quantifying the amount of hybridization complex, and comparing the amount of hybridization complex in the treated biological sample with the amount of hybridization complex in an untreated biological sample, where a difference in the amount of hybridization complex in the treated biological sample is indicative of toxicity of the test compound. (IV) is useful for producing (I) by recombinant techniques. (V) (a chimeric or single-chain antibody, Fab fragment, a F(ab')2 fragment, or a humanized antibody) is useful in a diagnostic test for a condition or disease associated with the expression of GCREC 1-16 in a biological sample. (V) is also useful for detecting (I) in a sample or purifying (I) from a sample. (VI) is useful for diagnosing a condition or disease associated with the expression of GCREC 1-16 in a subject. Preferably, the antibody in the composition is labeled. (C1), and (C2)

which comprises an agonist of (I) are useful for treating a disease or condition associated with decreased expression of functional GCREC 1-16. (C2) comprising antagonist of (I) is useful for treating a disease or condition associated with overexpression of (I). (VIII) is useful for generating a transcript image of a sample which contains polynucleotides, by labeling polynucleotides of sample and contacting them with elements of (VIII) to form hybridization complex and quantifying the expression of polynucleotides in the sample (all claimed). (I) and (II) are useful for the diagnosis, treatment or prevention of a cell proliferative (e.g., atherosclerosis, hepatitis), neurological (multiple sclerosis, Huntington's disease), cardiovascular (e.g., angina pectoris, heart failure, etc), gastrointestinal (such as anorexia, cholecystitis), autoimmune/inflammatory (Hashimoto's thyroiditis, rheumatoid arthritis), metabolic disorders (obesity, osteoporosis) and viral infections (all claimed).

ADMINISTRATION - The pharmaceutical compositions are administered by oral, intravenous, intramuscular, intraarterial, intramedullary, intrathecal, intraventricular, pulmonary, transdermal, or subcutaneous routes, etc. Dosages range from 0.1 mug-100,000 mug.

EXAMPLE - Incyte cDNAs were derived from cDNA **libraries** described in LIFESEQ GOLD database and as given in the specification, or cDNA **libraries** were constructed from cDNA obtained from RNA isolated by homogenizing certain tissues. Plasmids comprising cDNAs were recovered from Escherichia coli cells by in vivo excision, and purified. Incyte cDNAs recovered in plasmids were sequenced. The polynucleotide sequences derived from incyte cDNAs were validated by removing vector, linker and poly(A) sequences using algorithms and programs based on BLAST, dynamic programming, and dinucleotide nearest neighbor analysis. The Incyte cDNA sequences or translations of it were then queried against a selection of public databases. The Incyte cDNA sequences were **assembled** to produce full length **polynucleotide** sequences. The full length polynucleotide sequences were translated to derive the corresponding full length polypeptide sequences. Full length polypeptide sequences were subsequently analyzed by querying against databases such as the GenBank protein database, SwissProt, BLOCKS, PRINTS, DOMO, PRODOM, Prosite, and hidden Markov model (HMM)-based protein family databases such as PFAM. Putative G-protein **coupled** receptors were initially identified by running the Genscan gene identification program against public genomic sequence databases. Full length **polynucleotide** sequences were obtained by **assembling** Genscan-predicted coding sequences with Incyte cDNA sequences and/or public cDNA sequences using the above mentioned assembly process. Assembly of genomic sequence data with cDNA sequence data comprises stitched sequences and stretched sequences. Partial cDNAs assembled as described above were mapped to genomic DNA and parsed into clusters containing related cDNAs and Genscan exon predictions from one or more genomic sequences. Each cluster was analyzed using an algorithm based on graph theory and dynamic programming to integrate cDNA and genomic information, generating possible splice variants that were subsequently confirmed, edited, or **extended** to create a full length sequence. Sequence intervals in which the entire length of the interval was present on more than one sequence in the cluster were identified. Intervals thus identified were then stitched together by the stitching algorithm in the order that they appeared along their parent sequences to generate the longest possible sequence. Linkages between intervals which proceed along one type of parent sequence (cDNA to cDNA or genomic sequence to genomic sequence) were given preference over linkages which change parent type (cDNA to genomic sequence). The resultant stitched sequences were translated and compared by BLAST analysis to the genpept and gbpri public databases. Partial cDNAs assembled were queried against public databases using the BLAST program. The nearest GenBank protein homolog was then compared by the BLAST analysis to either Incyte cDNA sequences or GenScan exon predicted

sequences described above. A chimeric protein was generated by using the resultant high-scoring segment pairs (HSPs) to map the translated sequences onto the GenBank protein homolog. The GenBank protein homolog, the chimeric protein, or both were used as probes to search for homologous genomic sequences from the public human genome databases. Partial DNA sequences were therefore stretched or **extended** by the addition of homologous genomic databases. The resultant stretched sequences were examined to determine whether it contained a complete gene. The complete polynucleotide had a fully defined sequence of 2422, 1912, 1326, 3058, 1993, 1499, 2455, 2056, 999, 3429, 948, 939, 930, 1161, 948 or 924 nucleotides as given in the specification and encoded human G-protein **coupled** receptor had a fully defined sequence of 217, 578, 441, 797, 434, 339, 549, 188, 332, 948, 315, 312, 309, 309, 315 or 307 amino acids (S1-S16) as given in specification. (147 pages)

L169 ANSWER 29 OF 33 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-17048 BIOTECHDS
TITLE: New human regulatory **polynucleotide**, useful for treating disorders associated with protein truncation, particularly muscular dystrophy, and related peptides and antibodies;
vector-mediated dystrophin homolog gene transfer and expression in host cell for recombinant protein production, drug screening and gene therapy
AUTHOR: BARBER E
PATENT ASSIGNEE: IMPERIAL CANCER RES TECHNOLOGY LTD; BARBER E
PATENT INFO: GB 2368064 24 Apr 2002
APPLICATION INFO: GB 2000-1124 30 Sep 2000
PRIORITY INFO: US 2000-237079 30 Sep 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-429042 [46]
AB DERWENT ABSTRACT:
NOVELTY - **Polynucleotide** (I) comprising, or consisting of, a 137 bp sequence (1), fully defined in the specification, or its functional equivalents.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) **polynucleotides** (Ia) that hybridize to either strand of (I); (2) vector containing (I); (3) cell containing (I) or the vector of (b); (4) proteins and peptides encoded by (I); (5) protein (IIa) homologous with human dystrophin that is expressed on cell surfaces in vivo; (6) antibodies (Ab) specific for (II); and (7) method of screening for leukemia cells by analyzing DNA for presence of (1) or by detecting presence of (II).

BIOTECHNOLOGY - Preferred Nucleic Acid: (I) is at least 70% homologous with human dystrophin (hD), especially it includes a region of DNA that is homologous or identical with an hD sequence but has the inverted orientation. Particularly (I) is a 996 bp sequence (2), reproduced and designated apo-dystrophin-4, or its mutants or variants, can not be expressed to protein in an in vitro transcription/translation system in absence of (1) or its equivalent; has the translation of its mRNA regulated by (1) or its functional equivalent; (iv) encodes a protein able to bind CD33; (v) encodes a protein expressed on the cell surface in vivo and/or contains several stop codons. (Ia) is particularly antisense RNA. Preferred protein: (II) is expressed from (2) and has molecular weight about 50, 40 or 25 kD. Particularly it includes one of the peptide sequences MYPIMEYSCSDRN; YIYIGNLNVADTM or DDLGRAMESLVSVMTDEE. (II) is expressed on the surface of cells, e.g. leucocytes or in brain, muscle or placental tissues, and is particularly a heterodimer. Process: In method (7), the protein is detected by reaction with Ab. Preparation: A cDNA **library** from K562 cells, established in COS cells, was screened with an Fc-CD33 probe and 20 new cDNAs identified. One clone was sequenced to identify a sequence designated apo-dystrophin-4; it included

(1) as an inversion near the **3'-end**, and also included a **block** of stop codons. The inversion is a regulatory element that seems to allow read-through of the stop codons.

ACTIVITY - Muscular. No details of tests for activity are given.

MECHANISM OF ACTION - (1) is a regulatory element that controls expression (transcription and translation) of associated DNA, and may allow read-through of stop codons.

USE - (I) is used in gene therapy of diseases associated with truncation of proteins, particularly muscular dystrophy, but more generally (1) is a regulatory sequence used to control expression of any attached gene. Analysis of DNA for (I), or detection of proteins (II) encoded by (I), can be used to screen for leukemic cells and related diseases. Antibodies raised against (II) can be used therapeutically, to inhibit (II) activity, also to detect (II) in screening assays.

EXAMPLE - None given. (222 pages)

L169 ANSWER 30 OF 33 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-04912 BIOTECHDS
TITLE: Preparing a DNA molecule comprising an amplifiable region, useful for producing a genomic **library**, comprises subjecting the DNA molecules to primer extension/nick translation;
DNA primer extension, DNA probe and DNA array
AUTHOR: LANGMORE J P; MAKAROV V
PATENT ASSIGNEE: UNIV MICHIGAN
PATENT INFO: WO 2001090415 29 Nov 2001
APPLICATION INFO: WO 2000-US16264 20 May 2000
PRIORITY INFO: US 2000-206095 20 May 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-114290 [15]
AB DERWENT ABSTRACT:

NOVELTY - Preparing, (M1), a DNA molecule having an amplifiable region comprising subjecting the DNA molecules to nick translation comprising DNA polymerization and 5'-3' exonuclease activity to produce nick translate molecules, is new.

DETAILED DESCRIPTION - The method (M1) of preparing a DNA molecule having an amplifiable region comprises: (a) obtaining a DNA sample comprising DNA molecules having regions to be amplified; (b) attaching upstream adaptor molecules to ends of DNA molecules of the sample to provide a nick translation initiation site; (c) subjecting the DNA molecules to nick translation comprising DNA polymerization and 5'-3' exonuclease activity to produce nick translate molecules; and (d) attaching downstream adaptor molecules to the nick translate molecules to produce adaptor attached nick translate molecules. INDEPENDENT CLAIMS are also included for the following: (1) creating hybridization probes comprising preparing a labeled, amplified DNA; (2) shotgun sequencing of DNA; (3) constructing a genomic **library**; (4) preparing an unordered DNA **library**; (5) sequencing a BAC clone; (6) kits comprising amplifiable DNA; (7) an adaptor construct comprising: (a) a first domain comprising nucleotides that facilitate ligation of the construct to a nucleic acid; and (b) a second domain proximal to the first domain, comprising a site which facilitates the initiation of a nick translation reaction and a site that facilitates recombination, where ligation of the adaptor construct to a **polynucleotide** results in the only free 3' OH group capable of initiating a nick translation reaction within the second domain; (8) an adaptor construct comprising: (a) a first **oligonucleotide** comprising a phosphate group at the **5' end** and a **blocking** nucleotide at the **3' end**; (b) a second **oligonucleotide** comprising a **blocked 3' end**, a non-phosphorylated **5' end**, and a nucleotide sequence complementary to the 5' element of the first

oligonucleotide; and (c) a third **oligonucleotide** comprising a 3' hydroxyl group, a non-phosphorylated **5' end**, and a nucleotide sequence complementary to the 3' element of the first **oligonucleotide**; (9) kits comprising a DNA polymerase, nucleotide triphosphates, and an adaptor construct; (10) methods of recombining DNA molecules comprising recombining ends of adaptor attached template molecules in a dilute solution; and (11) a method of detecting a specific DNA sequence.

BIOTECHNOLOGY - Preferred Method: The ends of the DNA molecules are produced prior to the attachment of the upstream adaptor molecule, and by at least one restriction enzyme, by an endonuclease, by mechanical shearing, by a chemical, or a combination of these. The DNA polymerization step incorporates at least one modified nucleotide into the nick translate molecule, where the modified nucleotide is an exonuclease-resistant nucleotide and the adaptor attached nick translate molecules are separated. Separation is based upon size. The adaptor attached DNA molecule is denatured to separate the DNA. A single stranded nick translation product is separated from the DNA sample template strand. The DNA is subjected to nick translation for a specified period of time, and the nick translation product has a predictable length. The nick translate molecules are substantially similar in size. The upstream adaptor comprises a nick translation initiation site. The upstream adaptor further comprises a primer binding region, a hybridization domain, a ligation domain, a detection domain, an amplification domain, a recombination domain, or their combination. The downstream adaptor comprises a nick translation initiation site, and a hybridization domain, a ligation domain, a detection domain, an amplification domain, a recombination domain, or their combination. The upstream adaptor comprises at least a first and second upstream adaptor molecule construct having different primer binding regions. The method further comprises amplifying adaptor attached DNA molecules, and the amplified DNA is cloned into a vector, sequenced and separated. The method further includes creating a DNA **library**, which is an unordered DNA **library** or an ordered DNA **library**. Creation of the ordered DNA **library** further comprises recombination. The ordered DNA **library** comprises several nick translate molecules where nick translation of the nick translate molecules is carried out for different time. The ordered DNA **library** is further defined as a genomic ordered positional **library**. The adaptor attached nick translate molecules are amplified with primers complementary to the upstream adaptor molecule and the downstream adaptor molecule, or with a first primer specific to the upstream adaptor and a second primer specific to an internal sequence of the nick translate molecule. At least one of the primers used for amplification of the adaptor attached nick translate molecules is labeled. M1 further comprises amplifying the DNA molecules after adaptor attachment, hybridizing the amplified DNA to a microarray, and analyzing the hybridization patterns. The DNA sample is modified, preferably methylated. M1 further comprises initiating a second nick translation reaction at the upstream adaptor by subjecting the DNA molecules to nick translation using a DNA polymerase having 5'-3' exonuclease activity, and attaching second downstream adaptor molecules to the **5' end** of the molecules to produce adaptor attached nick translate molecules. The method also includes denaturing the adaptor attached nick translation product and separating the single stranded DNA, replicating the second strand of the adaptor attached molecule to form a double stranded product, subjecting the DNA molecules to nick translation using a DNA polymerase having 5'-3' exonuclease activity, to produce nick translate molecules, attaching additional downstream adaptor molecules to the nick translation initiation site of the nick translate molecules to produce adaptor attached nick translate molecule molecules. The affinity adaptor is ligated to the DNA molecules, and is used to separate DNA molecules. Preparing a DNA molecule having an amplifiable region may also comprise: (a) obtaining a DNA sample

comprising DNA molecules having regions to be amplified; (b) ligating an affinity adaptor to the proximal ends of the DNA molecules; (c) subjecting the affinity adaptor attached molecules to partial cleavage; (d) separating the affinity adaptor attached molecules; (e) attaching upstream adaptor molecules to ends of the affinity adaptor attached molecules to provide a nick translation initiation site;(f) subjecting the affinity adaptor attached molecules to nick translation comprising DNA polymerization and 5'-3' exonuclease activity; and (g) attaching downstream adaptor molecules to the nick translate molecules to produce adaptor attached nick translate molecules. Creating hybridization probes further comprises subjecting the adaptor attached nick translate molecules to recombination. The downstream adaptor is attached at the nick site, particularly the attachment is to the 5' strand of the nick site or to the 3' strand of the nick site. Recombination occurs at low DNA concentrations, and comprises digesting the DNA molecule with a first sequence-specific endonuclease; ligating both strands of an adaptor molecule to the sequence-specific termini of the template molecules; digesting the DNA molecules with a second sequence-specific endonuclease; incubating the DNA molecules at low concentration with large amount of T4 DNA ligase; concentrating the DNA molecules; initiating a nick-translation reaction for a controlled time; and attaching a down-stream adaptor. Alternatively, recombination comprises methylating the DNA molecules; ligating a first and second adaptor to the ends of the DNA molecule to form a recognition sequence, a single nick-translation initiation site, and a single Eco RI restriction recognition sequence within the recombination domain; activating the adaptors by incubation with a restriction enzyme or nuclease; incubating the DNA molecules at low concentration with a large mount of T4 DNA ligase; concentrating the DNA molecules; initiating a nick-translation reaction for a controlled time; and attaching a down-stream adaptor. The attached nick translate molecules are .5-500 kB in length. The DNA sample is cDNA, genomic DNA, cloned DNA, a BAC, a YAC, a cosmid or a large insert clone. The shotgun sequencing of DNA comprises preparing a DNA **library** by employing the method above; sequencing the **library** using primers specific for known loci to derive the sequence of adjacent unknown regions; recombining the DNA molecules after adaptor attachment; size separating the DNA molecules; and amplifying the DNA. The size separated DNA is distributed into the wells of a multi-well plate and the amplified DNA is sequenced and subsequently cloned into a vector. Restriction digestion is carried out with a frequent or infrequent cutter, and results in partial cleavage. The method further comprises attaching the upstream adaptor molecule to both the proximal and distal ends of the DNA molecules to create a circular product, where the initiation of nick translation occurs in the direction of the distal end of the nick translate molecule subjected to circularization, and the different internal regions of the nick translate molecules are exposed as distal ends. Nick translation is carried out on a DNA sample with several upstream adaptors or with several downstream adaptors in a single tube. Nick translation reaction proceeds through a known sequence on the DNA molecule, where PCR primers are constructed to recognize regions within the known sequence. PCR amplification of nick translate products occurs using a primers specific to the known sequence and a primer specific to an attached adaptor. The method further comprises circularizing the adaptor attached, nick translate product by incubating the adaptor attached, nick translate product with a linker **oligonucleotide** to form a nick site, and ligating the ends of the adaptor attached, nick translate product with a DNA ligase. The linker **oligonucleotide** is 20-200 bp long, and has a region complementary to the upstream adaptor and a region complementary to the downstream adaptor. In the method, the DNA molecules of the DNA sample are restricted with one or more restriction enzymes, the upstream adaptor molecules are attached at both ends of the restricted DNA molecules, nick translation is carried out from both upstream adaptors, and the ends of the DNA molecules are

recombined. The method further comprises separating the recombined molecules according to size. The restriction digestion is a partial digest. Each end of the DNA molecule is created with a different restriction enzyme. Alternatively, the DN A molecules of the DNA sample are restricted with an infrequent cutting restriction enzyme, the upstream adaptor molecules are attached at ends of the restricted DNA molecules, nick translation is carried out from the upstream adaptors, the nick translate molecules are partially restricted with a frequent cutter, internal adaptor molecules are attached at ends of the restricted DNA molecules, and nick translation is carded out from the internal adaptors, and the ends of the DNA molecules are recombined. The nucleotides integrated by nick translation are modified, and the modified nucleotides are exonuclease resistant and facilitates the differentiation of the nick translate product from the template strand. Constructing a genomic **library** comprises obtaining genomic DNA; fragmenting the genome to a desired size; attaching upstream adaptor molecules to ends of the fragmented genomic DNA molecules of the sample to provide a nick translation initiation site; subjecting the DNA molecules to nick translation comprising DNA polymerization and 5'-3' exonuclease activity; and attaching downstream adaptor molecules to the nick translate molecules to produce adaptor attached nick translate molecules. The nick translate molecules contain a known, kernel sequence, and are amplified with a primer or primers specific for the kernel sequence. The nick translate molecules are recombined, and recombination comprises ligating the upstream adaptor to the downstream adaptor. The recombined molecule further comprises a kernel sequence. The sequences adjacent to the kernel sequence are amplified. The adaptor attached nick translate molecules are inserted into a vector. The adaptor attached nick translate molecules are sequenced, and separated based on size. The upstream adaptor comprises a free 5' phosphate group. The adaptor attached nick translate molecule is recombined with a DNA ligase employing a linking **oligonucleotide** . The method further comprises incubating the linking **oligonucleotide** with the adaptor attached nick; translating the molecule to form a nick; and ligating the adaptor attached nick translate molecule with a DNA ligase. The ligase is preferably thermostable. Recombination is performed at a low DNA concentration. Preparing an unordered DNA **library** comprises obtaining a DNA sample comprising DNA molecules, cleaving the DNA molecules, attaching recombination adaptors to termini of the cleaved DNA molecules, subjecting file DNA molecules to nick translation comprising DNA polymerization and 5'-3' exonuclease activity, to produce nick translate molecules where the nick translation is initiated from both ends of the cleaved DNA molecules, and recombining the ends of the nick translate molecules produced. The recombined molecules are amplified, sequenced, and separated based on size. The adaptors are covalently joined by recombination. The recombined nick translate molecules are diluted prior to amplification. Dilution results in a reaction mixture with only a single DNA molecule. Sequencing is done by cycle sequencing employing a primer complementary to an adaptor and at least one or two base pairs adjacent to the adaptor. The amplified recombined nick translate molecules are cloned into a vector prior to sequencing. Sequencing a BAC clone comprises cleaving the BAC clone at a cos site with a lambda terminase, ligating an upstream adaptor to the 5' overhangs, partially cleaving the BAC clone with a frequently cutting enzyme, recombining the partially cleaved BAC clone, adding a homopolymeric tail to the **3 ' end** of the recombined product with terminal transferase, ligating an adaptor having a homopolymeric 3' single-strand overhang and a unique double strand sequence at the end to the homopolymeric tail, where the homopolymeric single-strand overhang is complementary to the homopolymeric tail, size separating the products, distributing the separated product into the wells of a microplate, amplifying the separated products with primers complementary to adaptor sequences such that products are formed which proceed in either a clockwise or

counterclockwise direction around the recombined molecule, ligating the amplified product into a cloning vector, and subsequently sequencing the amplified product. The adaptor attached nick translate molecules are distributed as an ordered microarray probed with complementary nucleic acid. Recombining DNA molecules comprises recombining ends of adaptor attached template molecules in a dilute solution. Recombination is further characterized by cleaving the DNA molecules with a first sequence-specific endonuclease; ligating an adaptor to the sequence-specific termini of the DNA molecule; cleaving the DNA molecules with a second sequence-specific endonuclease; incubating the DNA molecules under conditions to promote intra-molecule ligation of the DNA molecules; and concentrating the DNA molecules. The second sequence-specific endonuclease partially cleaves the DNA molecules. Recombination is further characterized by methylating the DNA molecules, attaching a first and second adaptor to the ends of the DNA molecules, where the adaptors comprise an activatable region, activating the adaptors by incubation with a restriction endonuclease to remove distal portion of the adaptors and creating sticky ends; incubating the DNA molecules under conditions to promote intra-molecule ligation of the DNA molecules; and concentrating the DNA molecules. Detecting a specific DNA sequence comprises separating adaptor attached nick translate molecules, and identifying the DNA sequence. The method further comprises hybridizing the adaptor attached nick translate molecules to a DNA microarray, and detecting the hybridization, where several of the specific DNA sequences are detected. The adaptor attached nick translate molecules are from humans, from several humans, or from several microorganisms. Preferred Kits: The kit comprises a DNA, preferably a genomic DNA isolated from a prokaryote, or a eukaryotic. The genomic DNA is preferably isolated from an animal selected from human, feline, canine, bovine, equine, porcine, caprine, murine, lupine, ranine, piscine and simian. The DNA may also be isolated from a dicotyledonous plant selected from tobacco, tomato, potato, sugar beet, pea, carrot, cauliflower, broccoli, soybean, canola, sunflower, alfalfa, cotton and Arabidopsis, or from a monocotyledonous plant selected from maize, rye, rice, turfgrass, oat, barley, sorghum, millet, and sugarcane. Preferred Construct: The adaptor construct further comprises a primer binding site, a hybridization domain, a detection domain, an amplification domain, a recombination domain, or a their combinations. The first domain comprises a nucleotide overhang. The site for initiation of a nick translation reaction comprises a single stranded region in an otherwise essentially double stranded molecule. The adaptor construct further comprises a domain that inhibits self ligation of the adaptor, and at least one degradable base which is degraded to create the free 3' OH group. The degradable base is deoxyribouracil. The **oligonucleotide** is 10-200 bases, and the second and the third **oligonucleotide** are 5-195 bases. The first **oligonucleotide** further comprises an additional 3' tail, a **3' end** protected from exonuclease activity, or one or more nuclease resistant nucleotide analogs. The third **oligonucleotide** comprises a **3' end** capable of initiating a nick translation reaction. The construct may further consist of several **oligonucleotides** which may be readily removed to expose a **3' terminus** of the adaptor, where each of the **oligonucleotides** comprise a nucleotide sequence complementary to a region of the first **oligonucleotide**. The removal of the **oligonucleotides** creates a site that facilitates recombination. The adaptor construct alternatively comprise a first **oligonucleotide** comprising a phosphate group at the **5' end** and a **blocking** nucleotide at the **3' end**; a second **oligonucleotide** comprising a **blocked 3' end**, a non-phosphorylated **5' end**, and a nucleotide sequence complementary to the 5' element of the first **oligonucleotide**; a third **oligonucleotide** comprising a

3' hydroxyl group, a non-phosphorylated **5' end**, and a nucleotide sequence complementary to the 3' element of the first **oligonucleotide**; and a fourth **oligonucleotide** comprising a 3' hydroxyl group, a non-phosphorylated **5' end**, and a nucleotide sequence complementary to the 3' element of the first **oligonucleotide**.

USE - The method is useful in producing DNA to be sequenced or amplified with specific regions for which the sequence is not known, and in producing a genomic **library**. The method is useful for sequencing internal regions of short templates using primary and secondary PENTAmers, and complement PENTAmers; sequencing large insert clones using ordered positional **libraries** of PENTAmers; genomic sequencing; determining gene positions; sequencing and re-sequencing; cDNA sequencing; diagnosing chromosomal rearrangements; detecting and identifying organisms and variants of organisms; and amplifying specific subsets of genomes.

ADVANTAGE - In contrast to PCR, which amplified DNA between 2 specific sequences, Primer Extension/Nick Translation can amplify DNA between 2 specific positions. Positional amplification by nick translation is fast and economical, because PENTAmer preparation can be multiplexed.

EXAMPLE - 0.8 mug primer extension/nick translation (PENT)-ready lambda DNA Bam HI templates were mixed with 500 fmol of 32P-labled PENT primer 5603 in 13.5 mul volume, heated to 70degreesC and allowed to cool slowly to room temperature for more than 2 hrs. PENT reaction was performed with wild type Taq DNA polymerase. Wild type Taq stock at 60 U/mul was always diluted 30x with Taq buffer before use. To conduct PENT reactions at different Taq DNA polymerase concentrations, 6 mixtures containing 5 mul of lambda DNA/Bam HI restriction fragments with ligated and activated nick-translation adaptor, 5 mul of 10x PCR buffer, 4mul 25 mM MgCl2, and 1, 1.5, 2, 3, 5 or 10 mul of Taq DNA polymerase and H2O to make a final volume of 49 mul were prepared in six 0.5 ml PCR tubes. Samples were pre-heated at 50degreesC for 5 min, and the PENT reactions were initiated by adding 1 mul of 2.5 mM dNTP solution to each tube. After 7 min incubation at 50degreesC, the reactions were terminated by adding EDTA and precipitated with ethanol. Products were separated on an alkaline 1% agarose gel. After electrophoresis, the gel is neutralized, electro-blotted onto ZetaProbe membrane and analyze with Molecular Dynamics 400A PhosphoImager and ImageQuant software. PENT products were detected as 1.4 kb band from 3-20 U of Taq DNA polymerase. (372 pages)

L169 ANSWER 31 OF 33 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-07485 BIOTECHDS
TITLE: Full length human hyperpolarized activated ion channel family proteins and **polynucleotides** useful in assays for measuring gene expression and for screening modulators of channel function which have therapeutic use; vector-mediated recombinant protein gene transfer and expression in host cell and use of antibody and database in diagnosis and in central nervous system, cardiovascular and reproductive diseasetherapy
AUTHOR: MORROW J A; DUNBAR D R; TOLAN D G
PATENT ASSIGNEE: AKZO NOBEL NV
PATENT INFO: WO 2001090142 29 Nov 2001
APPLICATION INFO: WO 2000-EP5959 24 May 2000
PRIORITY INFO: EP 2001-201344 12 Apr 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-097638 [13]
AB DERWENT ABSTRACT:

NOVELTY - A full length human hyperpolarized activated ion channel (I) of the HCN 1 subtype or their functional equivalents, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the

following: (1) a DNA sequence (II) encoding (I); (2) an expression vector (III) comprising a sequence of 2484 or 2673 bp as given in the specification or their functional equivalents or fragments; (3) antibodies (Ab) reactive with (I); (4) in vitro screening assay (M1) for the selection of compound that bind and/or influence a function of (I); and (5) compounds (IV) selected in (M1) useful in treatment of central nervous system disorders, cardiovascular dysfunction of the heart, and reproductive dysfunction and/or contraception related to Ih function in testes and spermatozoa.

BIOTECHNOLOGY - Preparation: The full-length DNA sequence for HCN1 is obtained using combined molecular biology and bioinformatics.

ACTIVITY - Cerebroprotective; Cardiant; Antiinfertility.

MECHANISM OF ACTION - Modulator of (I). No supporting data is given.

USE - (I) is useful for treating disorders such as human psychiatric and neurological dysfunction of central nervous system, cardiovascular dysfunction of the heart, and reproductive dysfunction and/or contraception related to Ih function in testes and spermatozoa and also useful for manufacture of medicament for the above mentioned diseases. (IV) is useful for manufacture of a medicament useful in treatment of the disorders. (M1) is useful for in vitro screening assay for the selection of compound that bind and/or influence a function of (I) (claimed). (I) is useful in assays for the measurement of gene expression. (II) is useful for deriving primers and probes for use in DNA amplification reactions to perform diagnostic procedures or to identify further, neighboring genes which also contribute to the expression of HCN1. (I) is useful in diagnostic assay for detecting clinical abnormalities or susceptibility to any of the above disorders or investigation of different clinical outcomes in response to medical treatment related to mutations in (II).

ADMINISTRATION - No administration details are given.

ADVANTAGE - In contrast with the prior art, the full length sequence of human HCN1 allows functional expression in in vitro systems for the identification of compounds modulating ion flux through the expressed channel. Unlike binding assay, assay based on channel function allows screening of compounds that interact with the channel as either **blockers** or openers. Binding assays give no such information, nor do they allow the identification of allosteric or use-dependent modulators of the channel. (M1) is quick and economic method to screen for therapeutic agents and is especially suited to be used for high throughput screening of numerous potential compounds.

EXAMPLE - Proprietary databases were screened by Basic Local Alignment Search Tool (BLAST) 2 for the presence of related human cDNA sequences using portions of the DNA sequence of the human HCN1 channel (Accession No AF064876) and mouse HCN1 (Accession number AJ225123). A single cDNA clone having a sequence (S1) of 1046 bp as given in the specification, was identified from a brain cDNA **library**, obtained and sequenced using an ABI prism 310 genetic analyzer. The clone encoded the **3' end** of human HCN1. The presence of a second splice variant was confirmed by polymerase chain reaction (PCR) using primers designed against the published sequence flanking either side of the 189 bp deletion identified in (S1) compared to the published sequence. The primers were 400 nM of Primer (P1) and (P2): 5'-TGCTGCAGCCCGGGGTCAACAAAT-3' (HCN1SPLICEA) and 5'-CTCCTGCCCCCTGCCTGAAG-3' (HCN1SPLICE B); or 5'-TCTACTACGACCCCGACCTC-3' (HCN1SPLICEC) and 5'-TGGCTCCCGACGACATCT-3' (HCN1SPLICED) PCR products of approximately 560bp (HCN1SPLICEA and HCN1SPLICEB) and 600bp (HCN1SPLICEC and HCN1SPLICED) were identified, purified and sequenced. This sequence had no deletion compared to the published sequence. Several independently derived clones were observed to contain a single nucleotide variant (a C to T transition) which results in change in amino acid (Ser to Phe) in a sequence of 346 amino acids as given in the specification. Primers were designated using the known human HCN1 sequence to attempt to obtain the **5' end** of HCN1 by rapid amplification of cDNA ends

(RACE)-PCR using Clontech SMART RACE cDNA amplification kit. SMART 5'-RACE-ready cDNA was synthesized by mixing 1mug of hippocampal polyA+ RNA with 2 muM 5'-RACE cDNA synthesis primer and 2 muM SMART II **oligonucleotide** (Clontech 5'-AAGCAGTGGTAACAACGCAGAGTACGGG-3'). A PCR product of approximately 300 bp was identified, purified and sequenced. RACE-PCR generated novel sequence information of 153 bp upstream of published human HCN1 sequence having a sequence of 153 bp as given in the specification, was used to screen by BLAST2 human public EST and genomic databases available from EMBL. A human genomic bacterial artificial chromosome (BAC) clone (RP11-398G9: accession number AC013384) that contained sequence similarity was identified from the high throughput genomic division of the EMBL release 62. The region corresponding to human HCN-1 was sequenced. This clone appeared to encode the **5' end** of human HCN-1 including the translation initiation codon. Several independently derived clones were found to have a single nucleotide variant (C to A transition) indicated in a sequence of 452 bp as given in the specification, resulting in a change of amino acid from Pro to Thr. The published human HCN1 sequence and the 5' sequence obtained by RACE-PCR was used to design primers to PCR the region of human HCN1 flanked by the RACE-PCR product and (S1) and covered by published sequence. 5'-TGCTGCAGCCCGGGGTCAACAAAT-3' (HCN1PCRA) 5'-GAGGCGGTGGGGGAGGCATAGTGG-3' (HCN1PCRB) A PCR product of approximately 1.9 kb was identified, purified and sequenced. Some independently derived clones were also found to contain a single nucleotide variation (C to T transition) indication in a sequence of 1873 bp as given in the specification. The full length sequence of HCN1 indicated that the cDNA consists of 248 bp open reading frame encoding an 827 amino acid channel.(63 pages)

L169 ANSWER 32 OF 33 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-07398 BIOTECHDS
TITLE: Novel G-protein **coupled** receptors and polynucleotides useful for diagnosis, treatment and prevention of disorders of cell proliferation, neurological, cardiovascular, metabolic disorders and viral infections; vector-mediated gene transfer, expression in host cell, antibody, transgenic animal, cDNA **library**, database, computer bioinformatic software and high throughput screening for recombinant protein production, drug screening anddisease gene therapy
AUTHOR: PATTERSON C; LU D A M; THORNTON M; LU Y; TRIBOULEY C M; GRAUL R; KHAN F A; GANDHI A R; WALIA N K; NGUYEN D B; YUE H; HAFALIA A; ELLIOTT V S; LAL P; REDDY R; KALLICK D A; TANG T Y; AU-YOUNG J
PATENT ASSIGNEE: INCYTE GENOMICS INC
PATENT INFO: WO 2001087937 22 Nov 2001
APPLICATION INFO: WO 2000-US16285 18 May 2000
PRIORITY INFO: US 2000-208861 2 Jun 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-089844 [12]
AB DERWENT ABSTRACT:
NOVELTY - An isolated polypeptide (I) of G-protein **coupled** receptor (GCREC) having a 372, 337, 346, 432, 240, 271, 276 or 408 residue amino acid sequence (S1), fully defined in the specification, a naturally occurring polypeptide comprising at least 90 % identity to (S1), a biologically active fragment of PP, and an immunogenic fragment PP, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an isolated polynucleotide (II) encoding (I); (2) a recombinant polynucleotide (III) comprising a promoter sequence operably linked to (II); (3) a cell (IV) transformed with (III); (4) a transgenic organism (V) comprising (III); (5) preparation of (I), comprising

culturing (IV) under expression conditions, and recovering the polypeptide; (6) an isolated antibody (VI) which specifically binds to (I); (7) an isolated polynucleotide (VII) comprising at least 60 contiguous nucleotides of (II); (7) detecting (M1) a target polynucleotide in a sample, where the target polynucleotide having a sequence of (II), comprising: (a) amplifying the target polynucleotide or their fragment using polymerase chain reaction; (b) detecting the amplified target polynucleotide or their fragment; and (c) optionally if present, determining the amount; (8) a composition (C1) comprising (I) or agonist of (I) identified by using (I), and an excipient; (9) a composition (C2) comprising antagonist of (I) identified by using (I) and an excipient; (10) a composition (C3) comprising (VI) and an excipient; (11) an polyclonal antibody (VIII) produced by using (I); (12) a monoclonal antibody (IX) produced by using (I); and (13) a composition (C4) comprising (VIII) or (IX) and a suitable carrier.

WIDER DISCLOSURE - A polynucleotide capable of hybridizing to (II) under stringency conditions, is disclosed as new.

BIOTECHNOLOGY - Preparation: (I) is prepared by culturing (IV) under conditions suitable for expression of (I), and recovering (I) so expressed. (VI) is produced by screening a Fab expression **library**, or a recombinant immunoglobulin **library**. Preferred Polynucleotide: (II) is preferably from a polynucleotide (P1) comprising a 2444, 1014, 1083, 1740, 3002, 965, 1617 or 1227 base pair sequence (S2), fully defined in the specification, a naturally occurring polynucleotide (P2) comprising a sequence at least 90 % identical to (S2), a polynucleotide (P3) complementary to polynucleotide of (P1) or (P2), and an RNA equivalent of (P1)-(P3). Preferred Antibody: (VI) is a chimeric antibody, single chain antibody, a Fab fragment, a F(ab')2 fragment or a humanized antibody.

ACTIVITY - Antiarteriosclerotic; Antiinflammatory; Hepatotropic; Antipsoriatic; Cytostatic; Anti-convulsant; Nootropic; Neuroprotective; Antiparkinsonian; Neuroleptic; Antianemic; Hypotensive; Cardiant; Antidiabetic; Antiulcer; Antidiarrheic; Laxative; Anti-HIV (human immunodeficiency virus); Anti-allergic; Dermatological; Antiasthmatic; Antigout; Antirheumatic; Antiarthritic; Immunosuppressive; Tranquilizer; Vulnerary; Ophthalmological; Anorectic; Osteopathic; Anti-bacterial; Fungicide; Virucide; Antiparasitic; Protozoacide; Nephrotropic. No biological data is given.

MECHANISM OF ACTION - Gene therapy; (I) modulator.

USE - (VII) is useful as a probe for detecting a target polynucleotide in a sample, where the target polynucleotide having a sequence of (II). (I) is useful for screening a compound for effectiveness as an agonist or antagonist of (I). The method comprises exposing a sample comprising (I) to a compound and detecting agonist or antagonist activity in the sample. (C1) and (C2) are useful for treating a disease or condition associated with decreased and overexpression of functional GCREC, respectively. (I) is also useful for screening a compound that specifically binds to (I). (I) is further useful for screening for a compound that modulates the activity of (I). (II) is useful for screening (M5) a compound for effectiveness in altering expression of a target polynucleotide. (VII) is useful as a probe for assessing toxicity of a test compound. (VI) is useful for diagnostic test for a condition or disease associated with the expression of GCREC in a biological sample. (I) is useful for preparing a polyclonal antibody with the specificity of (VI). (I) is further useful for making a monoclonal antibody with the specificity of (VI). (VI) is also useful for detecting a (I). (All claimed). (I) is useful for diagnosing, treating, preventing disorders which include cell proliferative disorders such as arteriosclerosis, hepatitis, myelofibrosis, psoriasis and cancer including adenocarcinoma, leukemia, lymphoma; neurological disorders such as epilepsy, ischemic cerebrovascular disease, Alzheimer's disease, Pick's disease, dementia, Parkinson's disease, ataxias, multiple sclerosis, bacterial and viral meningitis, Creutzfeldt-Jakob disease,

schizophrenic disorders, amnesia; cardiovascular disorders such as arteriovenous fistula, atherosclerosis, hypertension, vascular tumors, myocardial infarction, hypertensive heart disease, infective endocarditis, cardiomyopathy, myocarditis; gastrointestinal disorders such as dysphagia, peptic esophagitis, anorexia, nausea, emesis, peptic ulcer, cholelithiasis, diarrhea, constipation, acquired immunodeficiency syndrome (AIDS), hepatic encephalopathy; autoimmune/inflammatory disorders such as Addison's disease, allergies, spondylitis, amyloidosis, anemia, asthma, contact dermatitis, Crohn's disease, diabetes mellitus, emphysema, Goodpasture's syndrome, gout, Graves' disease, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, uveitis, viral, bacterial, fungal, parasitic, protozoal, and helminthic infections, and trauma; metabolic disorders such as diabetes, obesity and osteoporosis; or viral infections such as infection caused by viral agent classified as adenovirus, arenavirus, bunyavirus; and also in the assessment of effects of exogenous compounds on the expression of nucleic acid sequence of GPCR.

ADMINISTRATION - (II) is administered by a adenovirus-, or herpes-based gene therapy delivery system. C1, C2 or C3 is administered through oral, intravenous, intramuscular, intraarterial, intramedullary, intrathecal, intraventricular, pulmonary, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual or rectal routes. Dosage is 0.1-100000 micro-g upto a total dose of 1 g.

EXAMPLE - Incyte cDNAs were derived from cDNA **libraries** described in the LIFESEQ GOLD (RTM) database. Some tissue were homogenized and lysed in guanidinium isothiocyanate, while other were homogenized and lysed in phenol or in a suitable mixture of denaturants, such as TRIZOL (RTM). The resulting lysates were centrifuged over CsCl cushion or extracted with chloroform. RNA was precipitated from the lysates with either isopropanol or sodium acetate and ethanol, or by other routine methods. Phenol extraction and precipitation of RNA were repeated as necessary to increase RNA purity. Optionally, RNA was treated with DNase. For most **libraries**, poly(A)+RNA was isolated using oligo d(T)-**coupled** paramagnetic particles (Promega), OLIGOTEX (RTM) latex particles. Alternatively, RNA was isolated directly from tissue lysates using other RNA isolation kits, e.g. the POLY(A)PURE (RTM) mRNA purification kit. cDNA was obtained from RNA by Stratagene or synthesized and cDNA **libraries** were constructed with UNIZAP (RTM) vector system or SUPERSCRIPT (RTM) plasmid system. Reverse transcription was initiated using oligo d(T) or random primers. Synthetic **oligonucleotide** adapters were ligated to double stranded cDNA, and the cDNA was digested with the appropriate restriction enzyme or enzymes. For most **libraries**, the cDNA was size-selected (300-1000 base pair) using SEPHACRYL S1000 (RTM), SEPHAROSE CL2B (RTM), or SEPHAROSE CL4B (RTM) column chromatography or preparative agarose gel electrophoresis. cDNAs were ligated into compatible restriction enzyme sites of the polylinker of a suitable plasmid e.g. pBLUESCRIPT (RTM) plasmid. Recombinant plasmids were transformed into competent Escherichia coli cells including XL1-Blue. Then, plasmids were recovered from host cells by in vivo excision using the UNIZAP (RTM) vector system and were purified. Following precipitation, plasmids were resuspended in 0.1 ml of distilled water and stored, with or without lyophilization at 4 degrees C. Alternatively, plasmid DNA was amplified from host cell lysates using direct link polymerase chain reaction (PCR) in a high-throughput format. Host cell lysis and thermal cycling steps were carried out in a single reaction mixture. Samples were processed and stored in 384-well plates, and the concentration of amplified plasmid DNA was quantified fluorometrically using PICOGREEN (RTM) dye and a FLUOROSKAN II (RTM) fluorescence scanner. Incyte cDNA recovered in plasmids were sequenced. The Incyte cDNA sequences or their translations were then queried against a selection of public databases such as the GenBank primate, rodent, mammalian, vertebrate, and eukaryotic databases, and BLOCKS, PRINTS, DOMO, PRODOM, and hidden Markov model (HMM)-based protein family

databases such as PFAM. The queries were performed using programs based on BLAST, FASTA, BLIMPS, and HMMER. The Incyte cDNA sequences were **assembled** to produce full length **polynucleotide** sequences. Alternatively, GenBank cDNAs, GenBank expressed sequence tags (EST), stitched sequences, stretched sequences, or Genscan-predicted coding sequences were used to **extend** Incyte cDNA assemblages to full length. Assembly was performed using programs based on Phred, Phrap, and Consed, and cDNA assemblages were screened for open reading frames using programs based on GeneMark, BLAST, and FASTA. The full length polynucleotide sequences were translated to derive the corresponding full length polypeptide sequences.(135 pages)

L169 ANSWER 33 OF 33 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-07483 BIOTECHDS
TITLE: Neuregulin-2 polypeptide and polynucleotide useful for treating multiple sclerosis, spinal muscular atrophy, nerve injury, Alzheimer's disease, by increasing mitogenesis, survival, growth or differentiation of a cell; vector-mediated recombinant protein gene transfer for expression in host cell, antisense, transgenic animal model construction, antibody, liposome, DNA primer and polymerase chain reaction for use in cancer, cardiovasculardisease, muscle disease and central nervous system disease diagnosis, therapy and gene therapy
AUTHOR: MARCHIONNI M A
PATENT ASSIGNEE: CENES PHARM INC
PATENT INFO: WO 2001089568 29 Nov 2001
APPLICATION INFO: WO 2000-US16896 23 May 2000
PRIORITY INFO: US 2000-206495 23 May 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-097612 [13]
AB DERWENT ABSTRACT:

NOVELTY - A substantially pure neuregulin (NRG)-2 polypeptide (I) comprising or consisting of a sequence for human NRG-2alpha or NRG2beta (clone 2b7) of defined number of amino acids as given in the specification, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a substantially pure nucleic acid molecule (II) encoding (I); (2) a substantially pure nucleic acid molecule identical to (II); (3) a nucleic acid molecule having a sequence antisense to the coding stand sequence of (II) or their fragment; (4) a vector (III) comprising (II) operably linked to a promoter; (5) a cell comprising (III); (6) a non-human transgenic animal (IV) comprising (II); (7) a non-human animal (V) having a knockout mutation in one or both alleles encoding (I); (8) a cell from (V); (9) an antibody (Ab) specific to (I); (10) diagnosing (M1) an increased likelihood of developing a NRG-2-related disease or condition in a test subject, comprising analyzing nucleic acid molecules of subject to determine whether the subject contains a mutation in NRG-2 gene that encodes (I); and (11) a kit for the analysis of (I) of a test subject comprising Ab.

BIOTECHNOLOGY - Preparation: (I) is **prepared** by standard recombinant methods. Preferred **Polynucleotide**: (II) preferably comprises a nucleic acid sequence for human NRG-2alpha or NRG-2beta (clone 267) as given in the specification. Preferred Vector: (III) is preferably a gene therapy vector.

ACTIVITY - Vasotropic; Cardiant; Antiarteriosclerosis; Hypotensive; Neuroprotective; Nootropic; Antiparkinsonian; Antitumor.

MECHANISM OF ACTION - Gene therapy; Activator of an erbB receptor. No supporting data is given.

USE - (M1) is useful for diagnosing an increased likelihood of developing a NRG-2-related disease or condition in a test subject (Claimed). (I) is useful for increasing the mitogenesis, survival, growth

or differentiation of a cell, where the cell expresses an erbB receptor that is selective for (I), where the erbB receptor is preferably from erbB4 homodimer, an erbB2/erbB4 heterodimer and an erbB1/erbB3 heterodimer, and the cell is from neuronal cell; a neuronal progenitor cell; neuronal-associated cell preferably Schwann cell, astrocyte, oligodendrocyte, O-2A progenitor cell, glial cell, microglial cell, olfactory bulb ensheathing cell, or sensory organ cell; and a muscle cell preferably from myoblast, a satellite cell, a myocyte, a skeletal muscle cell, a smooth muscle cell, and a cardiac muscle cell. (I) is useful for stimulating mitogenesis of a glial cell. The method comprises contacting the cell with a recombinant (I), where the glial cell is from oligodendrocytes, microglia, myelinating glia, or olfactory bulb ensheathing cell, and glial cells in an adult; for inducing myelination of a neuronal cell by a glial cell comprising contacting glial cell with (I). (I) is useful for increasing the cardiomyocyte survival, proliferation, growth, or differentiation in a mammal preferably human, which has a pathophysiological condition which affects cardiac muscle, where the condition is cardiomyopathy (preferably degenerative congenital disease), ischemic damage, cardiac trauma or heart failure; or which has a condition affecting smooth muscle which include atherosclerosis, vascular lesion, vascular hypertension, and degenerative congenital vascular disease; or a patient with myasthenia gravis. (I) is useful for affecting cellular communication between a neuronal-associated cell and a neuronal cell in a mammal preferably human, where the (I) interacts with neuronal-associated cell resulting in the production of at least one neurotrophic agent by the neuronal-associated cell, the neurotrophic agent or agents affect the mitogenesis, survival, growth, differentiation, or neurite outgrowth of the neuronal cell, more preferably (I) affects cellular communication in the central nervous system or peripheral nervous system of a mammal preferably a human and the administering step comprises administering a purified (I)-producing cell. (I) is useful for the treatment or prophylaxis of a pathophysiological condition of the nervous system in a mammal preferably central nervous system or peripheral nervous system, where the condition is demyelination of nerve cells, damage or loss of Schwann cells or a neurodegenerative disorder; peripheral neuropathy preferably a sensory nerve fiber neuropathy or a motor fiber and a sensory nerve fiber neuropathy, where (I) brings out neural regeneration or neural repair; the condition is multiple sclerosis; amyotrophic lateral sclerosis, spinal muscular atrophy, nerve injury, Alzheimer's disease, Parkinson's disease, cerebellar ataxia, and spinal cord injury, and the administering step comprises administering a purified (I)-producing cell, which comprises a recombinant DNA sequence comprising (II) operably linked to a promoter. (I) is also useful for stimulating proliferation of a cell. Ab is useful for treatment of a tumor comprising inhibiting proliferation of a tumor cell preferably a glial tumor cell; for treating of neurofibromatosis by inhibiting glial cell mitogenesis which comprises inhibiting the binding of (I) to a receptor present on surface of a glial tumor cell in an individual with neurofibromatosis; for inhibiting proliferation of a cell; and for detecting (I) in a sample by contacting the sample with Ab, and assaying for binding of Ab to the polyeptide (claimed). In addition, Ab can be **coupled** to compounds, such as radionuclides and liposomes for diagnostic or therapeutic uses.

ADMINISTRATION - (I) and Ab are administered through parenteral, intravenous, intraarterial, subcutaneous, intramuscular, intracranial, intraorbital, ophthalmic, intraventricular, intracapsular, intraspinal, intracisternal, intraperitoneal, intranasal, inhalation to deep lung, aerosol, by suppositories, oral, or topical (e.g. by applying an adhesive patch carrying a formulation capable of crossing the dermis and entering the bloodstream). (II) is administered by viral vectors, as well as non-viral methods involving transfection in vitro by means of any standard technique, including calcium phosphate, DEAE dextran, electroporation, protoplast fusion, and liposomes. Dosage of (II) is

0.006-0.6 mg/kg.

EXAMPLE - A full length cDNA encoding neuregulin (NRG)-2alpha was identified from cerebellum. Multiple probes to various regions of NRG-2 coding sequences were designed based on rodent and human sequence data for cloning, mapping and sequence analysis. Approximately 400000 cDNAs from two human cerebellum gammat10 cDNA **libraries** were screened with an **oligonucleotide** probe: 5'-GCATCAACCAGCTCTCCTGC3' from the EGFL domain of NRG-2. 25 hybridization signals were detected; 20 of the phage clones corresponding to these signals were cloned and further analyzed by hybridization studies, physical mapping, and DNA sequencing. The results of these analyses were consistent with the existence of multiple structural variants (isoforms) among the human NRG-2 clones that were identified. Preliminary structural information on the clones was obtained by filter hybridization to phage plaques and restriction endonuclease analyses of the cDNA inserts. Polymerase chain reaction (PCR) studies, using internal primers, in pairs or in combination with flanking sequences, were used to obtain physical mapping data. The primers used were as follows: primer 1471:5'-GCATCAACCAGCTCTCCTGC-3'; primer 1494:5'-TGCGAACTGCTGACACCTGT-3'; primer 1527:5'-CCACCTTTTGAGCAAGTTCAG-3'; primer 1528:5'-GAGGTGGCTTATGAGTTCTTC-3'; primer 1531:5'-GGCCACCACACAGACGATG-3'. First, the insert sizes, which ranged from 0.8-3.3 kb (average size was roughly 1.7 kb) were analyzed. NRG-2 transcripts contained an EGFL domain and cytoplasmic sequences that exhibit much of the structural diversity of these polypeptides, and this specific internal region was focused on next to map the clones by PCR analysis. This analysis yielded four groups of products, and multiple clones were identified in each group. Therefore, the four groups (A-D) were likely to represent the **extent** of structural diversity in this region among the NRG-2 gene products in human cerebellum. Four clones (group A) gave no product in this experiment. This result was consistent with the data from hybridization experiments, which had shown that these clones lacked the sequence of the downstream primer (in the cytoplasmic domain). In the third experiment, the orientation of the clones was determined and the distance from the EGFL domain to the ends of the clones was estimated by using primers in the EGFL domain in combination with primers from flanking sequences in the phage arms. These studies, therefore enabled the segregation of the NRG-2 cDNAs into groups, and facilitated identification of potential full-length cDNAs encoding secretable isoforms of human NRG-2.(79 pages)

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L30          220 SEA FILE=CAPLUS ABB=ON  T4(L)(RNA OR RIBONUCLEIC)(L)LIGASE#/OBI

L32            1 SEA FILE=CAPLUS ABB=ON  L29 AND L30



L7         24949 SEA FILE=CAPLUS ABB=ON  3(1W)(END# OR TERMIN?)
L8         21724 SEA FILE=CAPLUS ABB=ON  5(1W)(END# OR TERMIN?)
L13        59979 SEA FILE=CAPLUS ABB=ON  OLIGONUCLEOTIDE#
L14        16723 SEA FILE=CAPLUS ABB=ON  POLYNUCLEOTIDE#
L29           59 SEA FILE=CAPLUS ABB=ON  DDUTP
L30          220 SEA FILE=CAPLUS ABB=ON  T4(L)(RNA OR RIBONUCLEIC)(L)LIGASE#/OBI

L36         1244 SEA FILE=CAPLUS ABB=ON  L14(L)(SYNTHES? OR PREP? OR ASSEMB?)/OB
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L39           10 SEA FILE=CAPLUS ABB=ON  L36 AND (L7 OR L8 OR L13) AND (L29 OR
                 L30)


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L50          11 SEA FILE=WPIDS ABB=ON  DDUTP OR DD UTP
L51          15 SEA FILE=WPIDS ABB=ON  T4(3A)(RNA OR RIBONUCLEIC OR RIBO
                NUCLEIC)(3A)LIGASE#
L52        1348 SEA FILE=WPIDS ABB=ON  L44(5A)(SYNTHE? OR PREP? OR ASSEMB?)
L53           1 SEA FILE=WPIDS ABB=ON  L52 AND (L50 OR L51)



L50          11 SEA FILE=WPIDS ABB=ON  DDUTP OR DD UTP
L51          15 SEA FILE=WPIDS ABB=ON  T4(3A)(RNA OR RIBONUCLEIC OR RIBO
                NUCLEIC)(3A)LIGASE#
L54           2 SEA FILE=WPIDS ABB=ON  L50 AND L51



L44       15218 SEA FILE=WPIDS ABB=ON  POLY NUCLEOTIDE# OR POLYNUCLEOTIDE#
L50          11 SEA FILE=WPIDS ABB=ON  DDUTP OR DD UTP
L51          15 SEA FILE=WPIDS ABB=ON  T4(3A)(RNA OR RIBONUCLEIC OR RIBO
                NUCLEIC)(3A)LIGASE#
L56       12711 SEA FILE=WPIDS ABB=ON  LIBRAR?
L57           1 SEA FILE=WPIDS ABB=ON  L44 AND (L50 OR L51) AND L56


=> s (l53 or l54 or l57) not (l42 or l165)   previously printed

L171          1 (L53 OR L54 OR L57) NOT (L42 OR L165)

=> fil biosis; d que l87; d que l88; d que l91

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CAS REGISTRY NUMBERS AND CHEMICAL NAMES (CNs) PRESENT
FROM JANUARY 1969 TO DATE.

RECORDS LAST ADDED: 25 December 2002 (20021225/ED)



L74           29 SEA FILE=BIOSIS ABB=ON  DDUTP
L75          163 SEA FILE=BIOSIS ABB=ON  T4(3A)(RNA OR RIBONUCLEIC OR RIBO
                NUCLEIC)(3A)LIGASE#
L87           0 SEA FILE=BIOSIS ABB=ON  L75 AND L74



L71        7751 SEA FILE=BIOSIS ABB=ON  POLY NUCLEOTIDE# OR POLYNUCLEOTIDE#
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L74          29 SEA FILE=BIOSIS ABB=ON  DDUTP
L88           0 SEA FILE=BIOSIS ABB=ON  L71 AND L74


L71        7751 SEA FILE=BIOSIS ABB=ON  POLY NUCLEOTIDE# OR POLYNUCLEOTIDE#
L75         163 SEA FILE=BIOSIS ABB=ON  T4(3A)(RNA OR RIBONUCLEIC OR RIBO
                NUCLEIC)(3A)LIGASE#
L90         472 SEA FILE=BIOSIS ABB=ON  L71(5A)(PREP? OR ASSEMB? OR SYNTHES?)
L91           2 SEA FILE=BIOSIS ABB=ON  L90 AND L75

=> s l91 not (l67 or l166)   previously printed
L172          2 L91 NOT (L67 OR L166)

=> fil biotechno; d que l119; d que l125; d que l126

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FILE LAST UPDATED: 17 DEC 2002       <20021217/UP>
FILE COVERS 1980 TO DATE.

>>>  SIMULTANEOUS LEFT AND RIGHT TRUNCATION AVAILABLE IN
     /CT AND BASIC INDEX  <<<


L106         57 SEA FILE=BIOTECHNO ABB=ON  T4(3A)(RNA OR RIBONUCLEIC OR RIBO
                NUCLEIC)(3A)LIGASE#
L107         15 SEA FILE=BIOTECHNO ABB=ON  DDUTP
L119          0 SEA FILE=BIOTECHNO ABB=ON  L106 AND L107


L103       1462 SEA FILE=BIOTECHNO ABB=ON  POLYNUCLEOTIDE#
L107         15 SEA FILE=BIOTECHNO ABB=ON  DDUTP
L125          0 SEA FILE=BIOTECHNO ABB=ON  L103 AND L107


L103       1462 SEA FILE=BIOTECHNO ABB=ON  POLYNUCLEOTIDE#
L106         57 SEA FILE=BIOTECHNO ABB=ON  T4(3A)(RNA OR RIBONUCLEIC OR RIBO
                NUCLEIC)(3A)LIGASE#
L108     128008 SEA FILE=BIOTECHNO ABB=ON  BLOCK? OR CAP####
L126          2 SEA FILE=BIOTECHNO ABB=ON  L108 AND L103 AND L106

=> s l126 not (l100 or l167)   previously printed
L173          1 L126 NOT (L100 OR L167)

=> fil biotechds; d que l148; d que l150; d que l151; d que l153

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FILE LAST UPDATED: 20 DEC 2002       <20021220/UP>

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L140       9591 SEA FILE=BIOTECHDS ABB=ON  POLYNUCLEOTIDE# OR POLY NUCLEOTIDE#

L145         10 SEA FILE=BIOTECHDS ABB=ON  DDUTP
L148          1 SEA FILE=BIOTECHDS ABB=ON  L140 AND L145



L140       9591 SEA FILE=BIOTECHDS ABB=ON  POLYNUCLEOTIDE# OR POLY NUCLEOTIDE#

L142       9007 SEA FILE=BIOTECHDS ABB=ON  LIBRAR?
L146         56 SEA FILE=BIOTECHDS ABB=ON  T4(3A)(RNA OR RIBONUCLEIC OR RIBO
                NUCLEIC)(3A)LIGASE#
L150          1 SEA FILE=BIOTECHDS ABB=ON  L140 AND L146 AND L142



L145         10 SEA FILE=BIOTECHDS ABB=ON  DDUTP
L146         56 SEA FILE=BIOTECHDS ABB=ON  T4(3A)(RNA OR RIBONUCLEIC OR RIBO
                NUCLEIC)(3A)LIGASE#
L151          1 SEA FILE=BIOTECHDS ABB=ON  L145 AND L146



L140       9591 SEA FILE=BIOTECHDS ABB=ON  POLYNUCLEOTIDE# OR POLY NUCLEOTIDE#

L146         56 SEA FILE=BIOTECHDS ABB=ON  T4(3A)(RNA OR RIBONUCLEIC OR RIBO
                NUCLEIC)(3A)LIGASE#
L152        715 SEA FILE=BIOTECHDS ABB=ON  L140(5A)(PREP? OR SYNTHES? OR
                ASSEMB?)
L153          4 SEA FILE=BIOTECHDS ABB=ON  L152 AND L146

=> s (l148 or l150 or l151 or l153) not (l138 or l168)   previously printed
L174          3 (L148 OR L150 OR L151 OR L153) NOT (L138 OR L168)

=> dup rem l170,l172,l173,l174,l171
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PROCESSING COMPLETED FOR L170
PROCESSING COMPLETED FOR L172
PROCESSING COMPLETED FOR L173
PROCESSING COMPLETED FOR L174
PROCESSING COMPLETED FOR L171
L175         13 DUP REM L170 L172 L173 L174 L171 (1 DUPLICATE REMOVED)
                ANSWERS '1-7' FROM FILE CAPLUS
                ANSWER '8' FROM FILE BIOSIS
                ANSWER '9' FROM FILE BIOTECHNO
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ANSWERS '10-12' FROM FILE BIOTECHDS
ANSWER '13' FROM FILE WPIDS

=> d ibib ab 1-13; fil hom

L175 ANSWER 1 OF 13 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 1
ACCESSION NUMBER: 1979:83229 CAPLUS
DOCUMENT NUMBER: 90:83229
TITLE: Studies on the **synthesis** of **polynucleotides**. **Synthesis** of a dodecaribonucleoside undecaphosphate and a hexadecaribonucleoside pentadecaphosphate
CORPORATE SOURCE: Shanghai Institute of Cell Biology, Collaboration Group Nucl. Acid Synth., Acad. Sin., Shanghai, Peop. Rep. China
SOURCE: Scientia Sinica (English Edition) (1978), 21(5), 687-97
CODEN: SSINAV; ISSN: 0582-236X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The synthesis of oligoribonucleotides by T4 RNA ligase and their identification are described. The products are a dodecaribonucleotide, CpGpGpA32pCpUpCpGpUpCpCpA, and a hexadecaribonucleotide, ApUpUpC32pCpGpGPA32pCpUpCpGpUpCpCpA, representing the **3'-end** nucleotide sequences of yeast alanine transfer RNA. Particular emphasis was placed on the conditions for the joining reaction with oligoribonucleotides of defined sequence as the substrates. CpGpGpA32pCpUpCpGpUpCpCpA was synthesized with 32pCpUpCpGpUpCpCpA as the donor, and CpGpGpA as the acceptor in rather low ratio of acceptor to donor. Both the yield and purity of the joining product were >90%. The 32pCpGpGpA32pCpUpCpGpUpCpCpA was used in turn as the donor, and ApUpUpC as the acceptor for further synthesis of the hexadecaribonucleotide with a yield of 60%.

L175 ANSWER 2 OF 13 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1995:656769 CAPLUS
DOCUMENT NUMBER: 124:30204
TITLE: Solid-phase **synthesis** of oligoribonucleotides using **T4 RNA ligase** and **T4 polynucleotide** kinase
AUTHOR(S): Vratskikh, L. V.; Komarova, N. I.; Yamkovoy, V. I.
CORPORATE SOURCE: Department Natural Sciences, Novosibirsk State University, Novosibirsk, 630090, Russia
SOURCE: Biochimie (1995), 77(4), 227-32
CODEN: BICMBE; ISSN: 0300-9084
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The concept of solid-phase synthesis of oligoribonucleotides using T4 RNA ligase and T4 polynucleotide kinase has been proposed and tested with model homo-oligoribonucleotides. The method consists of the immobilization of the first oligomer block at the **3'-terminus** on a solid support followed by a chain elongation in the 5'-direction with trinucleoside diphosphates using T4 RNA-ligase and phosphorylation using polynucleotide kinase. Hydrazides of Biogel P-300, Sepharose 4B and cellulose were tested as solid supports for immobilization of initial oligomers. The properties of supports were rated on reactivities of immobilized 5'-phosphorylated oligomers as phosphate donors in the solid phase reactions, hydrodynamical properties and capacity to eliminate donor mols. spontaneously during reactions. Hydrazide of Sepharose 4B appeared to be a more suitable support because of better hydrodynamic properties and highest reactivities of immobilized

donors. Satd. concns. of RNA ligase sand polynucleotide kinase and optimal time of joining reaction were detd. In a model expt. ApApA was twice attached to the immobilized hydrazide of Sepharose 4B donor (pA)6pAox. The yield of (Ap)12 was 25%.

L175 ANSWER 3 OF 13 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1990:94991 CAPLUS
DOCUMENT NUMBER: 112:94991
TITLE: Apparatus and modified dideoxy method for nucleotide sequencing and synthesis of labeled dideoxynucleotides
INVENTOR(S): Kikyoya, Tadashi
PATENT ASSIGNEE(S): Sekisui Chemical Co., Ltd., Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 27 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 63241356	A2	19881006	JP 1987-291265	19871118

PRIORITY APPLN. INFO.: JP 1986-278453 19861120

AB A modified dideoxy method for sequencing poly- and/or **oligonucleotides** comprises adding a single-chained nucleotide (primer) complementary to the test nucleotide, 4 types of unlabeled nucleoside triphosphates, and .gtoreq.1 type of differently-labeled nucleoside triphosphate derivs. [e.g., 2',3'-dideoxynucleoside-5'triphosphates (ddNTP) labeled with a colorant, fluorescent substance, chemiluminescent substance, or ligand-recognizing substance] to a reactor for a 1-step DNA synthesis reaction. An app. for sequencing combines the sequencing reactor with nucleotide sepn. and detection means, such as electrophoresis or HPLC means, and optical devices for monitoring the location and migration of the labeled nucleotides inthe electrophoretic or HPLC gel. X-RITC-labeled **ddUTP** (detectable at 615 nm), EITC-labeled ddCTP (detectable at 570 nm), TMRITC-labeled ddc.gamma.GTP (detectable at 601 nm), and FITC-labeled ddc.gamma.ATP (detectable at 520 nm) were prepd. and used in M13 mp DNA sequencing.

L175 ANSWER 4 OF 13 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1987:632010 CAPLUS
DOCUMENT NUMBER: 107:232010
TITLE: Enzymic incorporation of modified nucleosides into oligoribonucleotides
AUTHOR(S): Zhenodarova, S. M.; Klyagina, V. P.; Sedel'nikova, E. A.; Smolyaninova, O. A.; Soboleva, I. A.; Khabarova, M. I.; Gulyaeva, V. I.; Frolova, N. M.
CORPORATE SOURCE: Inst. Biol. Phys., Pushchino, USSR
SOURCE: Bioorganicheskaya Khimiya (1987), 13(8), 1037-44
CODEN: BIKHD7; ISSN: 0132-3423
DOCUMENT TYPE: Journal
LANGUAGE: Russian

AB The incorporation of modified nucleosides (tRNA components) and their analogs into **oligonucleotides** by a variety of RNases which differed in their substrate specificities, by polynucleotide phosphorylases, and by phage T4 RNA ligase was investigated. Pseudouridine, dihydrouridine, ribothymidine, 5-methylcytidine, inosine, and 6-methyladenosine could be incorporated by most of the RNases tested, including RNases Pb2, Pcl2, Pb1, Pch1, C2, T1, and A. 3-Methylcytidine and 4-acetylcytidine generally functioned as phosphate acceptors with the guanyl-specific RNases, whereas 1-methyladenosine was incorporated by RNase Pcl2. 7-Methylguanosine and 1-methylguanosine 2',3'-cyclophosphates could serve as phosphate donors with RNase Pb2. 6-Isopentenyladenosine

did not act as a phosphate acceptor for RNase Pb2.

L175 ANSWER 5 OF 13 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1981:457546 CAPLUS
DOCUMENT NUMBER: 95:57546
TITLE: Studies on the **synthesis** of **polynucleotides**. III. **Synthesis** of a decanucleotide fragment (36-45) of yeast alanine tRNA
CORPORATE SOURCE: Academia Sinica, Collaboration Group for Nucleic Acid Synthesis, Shanghai, 200031, Peop. Rep. China
SOURCE: Nucleic Acids Proteins, Proc. Symp. (1980), Meeting Date 1979, 254-9. Editor(s): Shen, Zhao-Wen. Sci. Press: Peking, Peop. Rep. China.
CODEN: 45RZAB
DOCUMENT TYPE: Conference
LANGUAGE: English
AB Since the trinucleotide Cpm1Ip.psi. was not an acceptor for T4 RNA ligase, another fragment was used in the synthesis of the decanucleotide fragment (36-45) of yeast tRNAAla. The tetranucleotide Cpm1Ip.psi.pG was reacted with pGpG to form Cpm1Ip.psi.pGpGpG, which was reacted with pApGpApGp to form the desired decanucleotide Cpm1Ip.psi.pGpGpGpApGpApG. All the steps involved the use of T4 RNA ligase. The specificity of the ligase reaction was demonstrated, including the ability of some nucleoside monophosphates (e.g., GpG) to ligate with pNp (N = nucleoside). Preheating the reaction mixt. at 60.degree. before addn. of enzyme increased the yield of the decanucleotide.

L175 ANSWER 6 OF 13 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1980:510061 CAPLUS
DOCUMENT NUMBER: 93:110061
TITLE: Specific labeling of **3' termini** of **RNA** with **T4 RNA ligase**
AUTHOR(S): England, T. E.; Bruce, A. G.; Uhlenbeck, O. C.
CORPORATE SOURCE: Dep. Biochem., Univ. Illinois, Urbana, IL, 61801, USA
SOURCE: Methods in Enzymology (1980), 65(Nucleic Acids, Pt. I), 65-74
CODEN: MENZAU; ISSN: 0076-6879
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The radiolabeling of RNA **3'-termini** by nucleoside 3',5'-bisphosphate-5'- 32P is described using T4 RNA ligase (EC 6.5.1.3). The nucleoside 3',5'-bisphosphates-5'-32P are prepd. from ATP-.gamma.-32P using T4 polynucleotide kinase.

L175 ANSWER 7 OF 13 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1968:503037 CAPLUS
DOCUMENT NUMBER: 69:103037
TITLE: **Polynucleotides**. LXXXVIII. Enzymic joining of chemically **synthesized** segments corresponding to the gene for alanine-tRNA
AUTHOR(S): Gupta, N. K.; Ohtsuka, E.; Sgaramella, V.; Buchi, H.; Kumar, A.; Weber, H.; Khorana, H. G.
CORPORATE SOURCE: Univ. of Wisconsin, Madison, WI, USA
SOURCE: Proc. Nat. Acad. Sci. U. S. (1968), 60(4), 1338-44
CODEN: PNASA6
DOCUMENT TYPE: Journal
LANGUAGE: English
AB DNA-joining enzymes, isolated from Escherichia coli and coliphage T4, catalyzed the in vitro joining of appropriate chem. synthesized deoxyribonucleotide segments of the gene for yeast alanine-tRNA, a DNA duplex of 30 nucleotides. These enzymes catalyzed the joining of short

oligonucleotides with free 5'-phosphate **end** groups.

L175 ANSWER 8 OF 13 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1983:214673 BIOSIS
DOCUMENT NUMBER: BA75:64673
TITLE: SYNTHESIS OF DECA NUCLEOTIDE AND HEXA NUCLEOTIDE CORRESPONDING TO THE FRAGMENTS 28-37 AND 37-42 OF YEAST INITIATOR VALINE TRANSFER RNA.
AUTHOR(S): ZHENODAROVA S M; KLYAGINA V P; SEDEL'NIKOVA E A; SMOLYANINOVA O A; KHABAROVA M I; MAISTRENKO V F; PUSTOSHILOVA N M
CORPORATE SOURCE: INST. BIOL. PHYS., ACAD. SCI. USSR, PUSHCHINO, USSR.
SOURCE: BIOORG KHIM, (1982) 8 (8), 1077-1083.
CODEN: BIKHD7.
FILE SEGMENT: BA; OLD
LANGUAGE: Russian
AB Decanucleotide .PSI.pCpUpGpCpUpUpIpApC and hexanucleotide CpApCpGpCpA corresponding to fragments 28-37 (where .PSI.-33 was replaced by uridine) and 37-42 of yeast tRNA1Val were synthesized. Initial oligonucleotide blocks .PSI.pCpU, pGpCpUpU, pIpApC, CpApC and pGpCpA were prepared enzymatically in the presence of RNases of different substrate specificity and polynucleotide phosphorylase. Fragments 28-30 and 31-34, 28-34 and 35-37, 37-39 and 40-42 were joined by **RNA-ligase T4**.

L175 ANSWER 9 OF 13 BIOTECHNO COPYRIGHT 2002 Elsevier Science B.V.
ACCESSION NUMBER: 1993:23105616 BIOTECHNO
TITLE: Covalent catalysis in nucleotidyl transfer. A KTDG motif essential for enzyme-GMP complex formation by mRNA **capping** enzyme is conserved at the active sites of RNA and DNA ligases
AUTHOR: Cong P.; Shuman S.
CORPORATE SOURCE: Program in Molecular Biology, Sloan-Kettering Institute,New York, NY 10021, United States.
SOURCE: Journal of Biological Chemistry, (1993), 268/10 (7256-7260)
CODEN: JBCHA3 ISSN: 0021-9258
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Vaccinia virus RNA **capping** enzyme, a heterodimer of 95- and 31-kDa subunits, catalyzes transfer of GMP from GTP to the 5'-diphosphate terminus of RNA via a covalent enzyme-guanylate intermediate. The GMP residue is attached to the 95-kDa subunit through a phosphoamide bond to the .epsilon.-amino group of a lysine residue. The amino acid sequence of the large subunit includes a lysine-containing motif, Tyr-X-X-X-Lys.sup.2.sup.6.sup.0-Thr-Asp-Gly, that is conserved in the RNA guanylyltransferases encoded by Shope fibroma virus and Saccharomyces cerevisiae. The KXDG motif is also encountered at the sites of covalent adenylylation of bacteriophage **T4 RNA ligase** and mammalian DNA ligase I (Thogerson, H. C., Morris, H. R., Rand, K. N., and Gait, M. J. (1985) Eur. J. Biochem. 147, 325-329; Tomkinson, A. E., Totty, N. F., Ginsburg, M., and Lindahl, T. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 400- 404). We find that conservative amino acid substitutions at three out of four positions within the KTDG sequence of vaccinia **capping** enzyme either prevent or strongly inhibit enzyme-guanylate formation. The conserved motif is therefore an essential component of the guanylyltransferase domain. Lys.sup.2.sup.6.sup.0 is implicated as the active site. Comparison of the sequences of **capping** enzymes and **polynucleotide** ligases from diverse sources suggests that KX(D/N)G may be a signature element for covalent

catalysis in nucleotidyl transfer.

L175 ANSWER 10 OF 13 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 1991-11468 BIOTECHDS
TITLE: **RNA-ligase** of bacteriophage **T4**.
VIII. A solid-phase enzymic synthesis of oligoribonucleotides;
oligonucleotide **synthesis** using phage **T4** **polynucleotide**-kinase and **RNA-ligase**
AUTHOR: Mudrakvoskaya A V; Yamkovoy V I
LOCATION: Novosibirsk State University, Novosibirsk, USSR.
SOURCE: Bioorg.Khim.; (1991) 17, 6, 819-22
CODEN: BIKHD7
DOCUMENT TYPE: Journal
LANGUAGE: Russian
AB A method for oligoribonucleotide synthesis was developed which involved fixing the donor at the 3'-end on a water-insoluble matrix, and subsequent extension of the nucleotide chain in the 5'-direction by 3 units per cycle. The cycles comprised: (a) phosphorylation of the donor using phage T4 polynucleotide-kinase and ATP; (b) washing; (c) internucleotide condensation using RNA-ligase and trinucleoside diphosphate; and (d) washing. Biogel P-300 was used as a matrix for fixing the donor, (pA)7, oxidized with sodium periodate. Up to 92% of the donor (pA6)pAox was fixed on the hydrazide of Biogel P-300. The RNA-ligase-catalyzed addition of acceptor ApApA to the donor (pA6)pAox was conducted at 30 deg for 24 hr. The donor was extended by 2 nucleotides after incubation with RNA-ligase and ApApA. The product, (Ap)9, was recovered in 50% yield. The oligoriboadenylates were eliminated from the hydrazide of Biogel P-300 with methylamine. (16 ref)

L175 ANSWER 11 OF 13 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 1984-00465 BIOTECHDS
TITLE: Enzymatic synthesis of oligonucleotides corresponding to the 3'-terminus of influenza virus RNA;
purification and sequence determination
AUTHOR: Zhenodarova S M; Klyagina V P; Sedelnikova E A; Smolyaninov O A; Khabarova M I; Belova E N
LOCATION: Institute of Biological Physics, Academy of Sciences of the USSR, Pushchino, USSR.
SOURCE: Bioorg.Khim.; (1983) 9, 10, 1382-87
CODEN: BIKHD7
DOCUMENT TYPE: Journal
LANGUAGE: Russian
AB The dodecanucleotide, CpCpUpGpCpUpUpUpUpGpCpU, was synthesized from the trinucleoside diphosphates, GpCpU and CpCpU, and the trinucleotides, pGpCpU and pGpCpUp, with the aid of **RNA-ligase** from **T4** phage. These initial blocks were obtained in 2 stages by means of specific ribonucleases and **polynucleotide** -phosphorylase. CpCpU was **synthesized** by using **polynucleotide**-phosphorylases from Escherichia coli and Micrococcus luteus. After enzyme inactivation the reaction mixture was separated on a DEAE-Sephadex column. Peaks corresponding to the individual components were desalted on Sephadex G-15 and subjected to paper chromatography. In order to determine the nucleotide sequence, /5(+)-32P/pCp was attached to the dodecanucleotide, treated with E.coli phosphomonoesterase, with the aid of RNA-ligase, and the dodecanucleotide was subjected to direct chemical sequencing. After the fragments formed had been separated by polyacrylamide gel electrophoresis, an autoradiogram was obtained which confirmed to dodecanucleotide structure. (13 ref)

L175 ANSWER 12 OF 13 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI

ACCESSION NUMBER: 1982-04561 BIOTECHDS
TITLE: Enzymatic synthesis of a 21-nucleotide coat protein binding fragment of R17 ribonucleic acid;
using **polynucleotide**-phosphorylase to **prepare** short oligomers and **T4 RNA-ligase** to join them
AUTHOR: Krug M; De Haseth P; Uhlenbeck O C
LOCATION: Department of Biochemistry, University of Illinois, Urbana, Illinois 61801, USA.
SOURCE: Biochemistry; (1982) 21, 4713-20
CODEN: BICHAW
DOCUMENT TYPE: Journal
LANGUAGE: English
AB An oligoribonucleotide with a sequence identical with the phage R17 replicase initiator region was synthesized. The 21-nucleotide fragment corresponding to R(-17 - +4), whre +1 is the first nucleotide of the replicase gene, was **synthesized** totally enzymatically, employing **polynucleotide**-phosphorylase (EC-2.7.7.8) to **prepare** short oligomers and **T4 RNA-ligase** (EC-6.5.1.3) and polynucleotide-kinase to join them. A branched synthetic protocol allowed the use of approximately equivalent amounts of the 6 starting oligomers and the combination of the starting oligomers was dicatated by the efficiency of their **synthesis** with **polynucleotide**-phosphroylase and the expected yields of RNA-ligase reactions based on previous studies with model compounds. The synthetic scheme involved 5 different RNA-ligase reactions to join 6 oligomers. The purity of the resultant product was demonstrated by chromatography, wandering spot sequencing procedure, formation of a unique Up(3'-5')U internucleotide linkage in the final ligase step and finally the determination of the 3' and 5' termini. (33 ref)

L175 ANSWER 13 OF 13 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 2001-529099 [58] WPIDS
DOC. NO. CPI: C2001-157765
TITLE: Detecting and/or quantitating nucleic acid in sample by using nucleic acid template dependent enzyme in combination with random primer to generate enzymatic product which incorporates binding and detectable species.
DERWENT CLASS: B04 D16
INVENTOR(S): CORCORAN, M V; HEROUX, J A; RAO, S M
PATENT ASSIGNEE(S): (CORC-I) CORCORAN M V; (HERO-I) HEROUX J A; (RAOS-I) RAO S M
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
US 2001014446	A1	20010816	(200158)*		15

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
US 2001014446	A1	US 1998-23483	19980213

PRIORITY APPLN. INFO: US 1998-23483 19980213
AB US2001014446 A UPAB: 20011010
NOVELTY - Detecting and/or quantitating (M) nucleic acid in a sample (S), comprising mixing a random primer having a detectable species (DS) with (S), adding a nucleotide triphosphate (NTP) having a binding species and optionally NTP, adding a nucleic acid polymerase, incubating the mixture,

contacting the mixture with a solid phase (SP) and detecting and/or quantitating DS bound to SP, is new.

DETAILED DESCRIPTION - In (M), the random primer of at least four nucleotides, has a binding species and optionally a detectable species. NTP has a detectable species and a label. (M) optionally involves adding a nucleic acid ligase, and optionally a nucleic acid polymerase, before addition of NTP. The mixture is incubated under conditions which allow the nucleic acid polymerase or nucleic acid ligase to be active.

An INDEPENDENT CLAIM is also included for a kit (I) comprising:

(a) a vial containing a random primer at least four nucleotides in length having a detectable species, and containing at least one NTP having a binding species and optionally one NTP, a vial containing a nucleic acid polymerase, and a vial containing a solid phase; or

(b) a vial containing a random primer at least four nucleotides in length having a binding species, and containing at least one NTP having a detectable species and optionally one NTP, a vial containing a nucleic acid polymerase, and a vial containing a solid phase.

USE - (M) is useful for detection and/or quantitation of nucleic acid in a sample (claimed).

ADVANTAGE - (M) is less technically challenging and labor intensive compared to assays currently used for detection of 10 pg amounts of DNA. (M) has a greater dynamic range for detection of DNA (5-10000 pg) at a 60 minute reaction time than any of the current assays. (M) detects DNA larger than 100 base pairs regardless of base sequence or species of DNA. Generation of the signal in (M) does not require radioactivity or incubation with a single amplifier or enzyme substrate. (M) recounts samples initially determined to be off the range of the standard curve. (M) improves the speed and accuracy for determining the amount of DNA in a sample, and the results are generated faster due to the ease of the protocol and short incubation time of the enzyme reaction.

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